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A Review of Approaches to Assess Survival of Released Catch from Canadian Large Pelagic Longline Fisheries

Examen de méthodes d'évaluation de la survie des captures remises à l'eau dans les pêches canadiennes de gros poissons pélagiques à la palangre

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ABSTRACT

To address part of the Terms of Reference for a Regional Advisory Process (RAP) meeting held July 11-12 entitled "Incidental Catch in Canadian Large Pelagics Fisheries", we review methods for assessing post-release mortality in marine fisheries. The methods can be grouped into five categories, including confinement, field observations, conventional tagging, telemetry, and physiological correlates of mortality. We recommend best practices for determining post-release survival in the Canadian pelagic longline fishery, focusing on the seven species of particular interest to the RAP: bluefin tuna (*Thunnus thynnus*), porbeagle (*Lamna nasus*), shortfin mako (*Isurus oxyrinchus*), blue shark (*Prionace glauca*), leatherback sea turtles (*Dermocheyls coriacea*), loggerhead sea turtles (*Caretta caretta*), and swordfish (*Xiphias gladius*). Of the currently available methods, we conclude that a combination of field observations using standardized release codes validated with telemetry offers the most informative results.

RÉSUMÉ

Pour répondre en partie au cadre de référence d'une réunion du Processus consultatif régional (PCR), tenue les 11 et 12 juillet 2011, portant sur les captures accessoires dans les pêches canadiennes de grands poissons pélagiques, nous examinons les méthodes d'évaluation de la mortalité après la remise à l'eau dans les pêches maritimes. Nous groupons les méthodes en cinq catégories, soit le confinement, les observations sur le terrain, l'étiquetage classique, la télémétrie et les corrélats physiologiques de la mortalité. Nous recommandons des pratiques exemplaires pour déterminer le niveau de survie après la remise à l'eau dans les pêches canadiennes de poissons pélagiques à la palangre, en particulier des sept espèces ciblées par le PCR, soit le thon rouge (*Thunnus thynnus*), le requin-taupe commun (*Lamna nasus*), le requin-taupe bleu (*Isurus oxyrinchus*), le requin bleu (*Prionace glauca*), la tortue luth (*Dermocheyls coriacea*), la caouanne (*Caretta caretta*) et l'espadon (*Xiphias gladius*). Des méthodes disponibles, nous concluons qu'une combinaison d'observations sur le terrain reposant sur des codes normalisés de remise à l'eau validés par télémétrie donne les résultats les plus informatifs.

INTRODUCTION

A considerable proportion of commercially-captured fish are released due to harvest restrictions (i.e., number and size limits) and catch of non-target species (Alverson et al. 1994, Kelleher 2005, Davies et al. 2009). In most cases, the fate of released fish remains unknown. Quantifying post-release survival of discarded catch has therefore been an ongoing challenge for fisheries management. Mortality of discarded fish may have serious economical and ecological consequences, as it represents a waste of natural resources, and exacerbates fishing pressure on commercial fish stocks, as well as on rare and endangered species (Crowder and Murawski 1998, Hall et al. 2000, Harrington et al. 2005). In addition, failure to incorporate post-release mortality into stock assessment models results in underestimates of fishing mortality, which in turn reduces the accuracy of the abundance and projected catch estimates, and may undermine the efficacy of conservation measures, such as size limits (Coggins et al. 2007).

Survival of discarded catch is influenced by multiple factors including environmental conditions (i.e. depth and temperature), biological characteristics of the species, technical factors (i.e. fishing practices and gear used; Davis 2002, Bartholomew and Bohnsack 2005, Broadhurst et al. 2006) and the handling of the animal. Furthermore, the fate of released fish is further complicated by the fact that gear encounter effects can range from behavioural impairment to immediate, short-term or delayed mortality (Crowder and Murawski 1998, Donaldson et al. 2008). Arlinghaus et al. (2007) produced a schematic (Fig. 1) that illustrates both the short and longer-term impacts of the process of capture, handling and release on subsequent survival, and the potential damages that can occur along with the biological end-points (i.e. injury, sub-lethal stress, mortality). This figure indicates the range of pathways that can lead to negative outcomes as well as highlighting the lack of information on some of the longer-term potential impacts of the capture process. From an applied point of view however, it highlights the two major components of fishing mortality: capture mortality, which is apparent when the (dead) fish is brought aboard the fishing vessel (i.e. hooking mortality), and post-release mortality, which can only occur after the fish is released.

Quantifying post release survival is no easy task and represents a large source of uncertainty in population assessments of commercially valuable species and non-target species of conservation concern (Alverson et al. 1994, Hueter et al. 2006, Moyes et al. 2006). Incorporation of reliable estimates of post-release mortality into stock assessments and catch projections would improve the accuracy and predictive value of those assessments. In addition, there is a need to better understand variables that influence survival, as they are integral to the development of mitigation measures to either reduce by-catch or limit post-release mortality (Richards et al. 1995, Hall and Mainprize 2005).

To address part of the Terms of Reference for a Regional Advisory Process (RAP) meeting held July 11-12 entitled “Incidental Catch in Canadian Large Pelagics Fisheries”, we review methods for assessing post-release mortality in other fisheries. We recommend best practices for determining post-release survival in the Canadian pelagic longline fishery, focusing on the seven species of particular interest to the RAP: bluefin tuna (*Thunnus thynnus*), porbeagle (*Lamna nasus*), shortfin mako (*Isurus oxyrinchus*), blue shark (*Prionace glauca*), leatherback sea turtles (*Dermochelys coriacea*), loggerhead sea turtles (*Caretta caretta*), and swordfish (*Xiphias gladius*). Within each category of approaches, we highlight work that has occurred with the Maritimes Region.

AVAILABLE APPROACHES FOR DETERMINING POST-RELEASE SURVIVAL

Numerous approaches have been developed to assess post release survival of discarded catch; these fall into five general categories, including confinement studies, condition or vitality data from field observations, conventional tagging, electronic tagging, and biochemical correlates with mortality. Each approach is critically evaluated in the following sections, the aim being to suggest best practices for estimating post-release mortality in the Canadian Maritimes pelagic longline fishery.

Confinement Methods

Holding studies, in which fish caught during commercial (or simulated commercial) fishing operations are handled according to regular practice then monitored in tanks or sea cages, have been used to assess the survival rate of discarded catch from various fisheries. This approach has provided some measure of survival rates and identified important factors that influence mortality for several commercial and non-commercial species. For instance, several studies have reported a size-related influence on survival rates, with larger fish having a higher survival rate (Neilson et al. 1989, Milliken et al. 1999, Broadhurst et al. 2006). However, there are considerable drawbacks associated with this approach. Confinement is not practical for large migratory species targeted by pelagic longline fisheries (Post et al. 1997, Skomal 2007). As a result, studies using this approach have focused on relatively small species; particularly those captured using towed gear (Broadhurst et al. 2006). Furthermore, due to the high cost of vessel time and personnel, confinement studies have been limited to short term examination of post-release mortality. Therefore, it precludes factors that could cause delayed mortality such as predation due to post gear impairment (Ross and Hokensen 1997, Milliken et al. 1999, Davis 2002). In addition, several studies have shown that confinement in itself can cause mortality through the propagation of infection due to overcrowding or additive stress associated with holding fish (Neilson et al. 1989, Mandelman and Farrington 2007).

Broadhurst et al. (2006) reinforced those concerns, noting that irrespective of whether experiments have been done in the field or laboratory, their utility in terms of estimating unaccounted fishing mortality depends on several criteria. These include (i) the use of appropriate controls, (ii) sufficient duration of monitoring, and (iii) the ability of captive conditions to be representative of the actual experience of the fish following capture and release. Controlling treatment effects are an inherent prerequisite to the attribution of causality in experimental designs (Underwood 1997). Various control groups, typically comprising organisms collected from passive gears, are required to isolate any confounding influences associated with the handling and confinement practices (Main and Sangster 1988, Suuronen et al. 1996b, Mensink et al. 2000). Any control-organism mortalities can then be used to adjust the estimates of treatment effects. Broadhurst et al. (2006) noted that the potential for mortalities to controls are real, with most studies recording deaths for control animals during the duration of the experiment (Suuronen et al. 1995, Suuronen et al. 1996a, 1996c, Mensink et al. 2000, Bergmann and Moore 2001a, 2001b). Despite the clear need for adequate controls as part of valid hypothesis testing, severe logistical constraints associated with their collection and housing have meant that these are rarely used in most experiments examining the mortality of discards. Appropriate controls are likely to be particularly problematic for the large pelagic longline fishery, since the logistical constraints in developing suitable controls is likely to be particularly difficult.

Concerning the appropriate duration of monitoring of fish held in captivity, Wassenberg and Hill (1993) designed an experiment to establish an appropriate duration for monitoring the mortality of subtropical fish and invertebrates. Based on a general plateau in deaths at 3 days, they suggested that 4 days monitoring was adequate. However, in subsequent work, Wassenberg et al. (2001) acknowledged that species-specific variability in susceptibility to various trauma associated with catch-and-discarding mechanisms means that some individuals can suffer considerable protracted mortality. The potential for delayed deaths clearly illustrates the benefits of using adequate controls to differentiate between treatment and experimental effects. In a review of the results of 88 peer-reviewed papers that studied post-release survival using captive animals, only a few studies had a holding duration of 30 days or longer (Broadhurst et al. 2006).

While a lack of appropriate controls increases the probability of over-estimating mortality, other factors associated with captivity and the inability to fully mimic conditions in the natural environment tend to bias mortality estimates in the opposite direction. For example, fish may be held at higher densities in captivity than might be expected in the wild, which might lead to increased stress or disease. Temperature and oxygen conditions in a holding tank seldom mirror those available to, or sought by, free-swimming fish, even where a flow-through water supply is available. Some workers have attempted to circumvent this problem by holding recovering haddock in individual submerged cages in the natural environment (Main and Sangster 1988), but such approaches are laborious and not practical for larger species. In particular, the exclusion of predators that might normally prey on fish that are damaged or stressed by capture implies that mortality estimates of confined fish may be greatly underestimated.

Unlike leatherback turtles, large pelagic sharks or teleost fish, loggerhead turtles have been successfully held in captivity to determine the long-term physiological effects and mortality levels associated with fishing injuries and release conditions (Casale et al. 2008). While such research will underestimate mortalities from depredation, post-capture mortalities resulting from secondary infections from hooking injuries or from ingestion of branchlines occurred within 36 days. These observations do not mimic post-release conditions but do provide direct evidence of the physiological effects associated with particular hooking injuries and release conditions. Post-hooking mortality levels were higher than estimated previously (Casale et al. 2008). A study of post-release mortality of deeply-hooked loggerhead sea turtles caught in the Spanish swordfish fishery found mortality rates of 20-30% (Aguilar et al. 1992). This was a small scale study of 38 captive turtles, from which the hooks were not removed. No control animals were held and no necropsies were performed to determine cause of death.

In light of these limitations, the confinement of fish caught during commercial or simulated commercial fishing operation is not a recommended method for determining the fate of discarded catch from pelagic longline fisheries.

Condition or Vitality Data from Field Observations

In many fisheries, including pelagic longline fisheries, trained at-sea observers collect data on fishing effort, catch, environmental conditions, fishing practices and condition of discards. In this case, post-release mortality estimates are derived from condition or vitality data collected by at-sea observers prior to discard. The number of levels of condition varies, depending largely on the ability to make detailed observations of the specimens caught, and may be limited to observations of individuals that have died on the fishing gear. In the Canadian pelagic longline fishery where fish are assessed for retention/release while the individuals are alongside the vessel, the opportunities to collect detailed information on condition are limited. In other fisheries where the fish are brought on board, different observations are possible. For example, Benoit et al. (2010) working on groundfish fisheries in the Gulf of St. Lawrence, described a four level ordinal condition scale (Table 2), conducted concurrent with a captivity study which illustrated that the observers' assessment of condition compared well with subsequent survival in onboard tanks (Table 3). In this study, it is interesting to note that a significant fraction of the sculpins and rays classified by observers as moribund actually survived at least 48 h.

The International Pacific Halibut Commission (IPHC) had used three categories of condition (excellent, poor, and dead), which were defined by a series of criteria related to injuries and physical response to stimuli (Williams and Wilderbuer 1995). These criteria were subjective and open to interpretation by the observers (Kaimmer and Trumble 1998). Kaimmer and Trumble (1998) also found inadequacies in the three-level approach of categorizing condition classes. Those authors found that based on tagging studies, the survival rates of the three classes were 97, 76 and 26% for the excellent, poor and dead categories, respectively. The authors concluded that the term “dead” was a misnomer. In response to those issues, the IPHC has developed a more elaborate scheme for grading condition, which was considered to be more objective than the previous approach (Table 4; Trumble et al. 2000).

Canada has had a long-standing program of fisheries observers operating within its Atlantic waters. The international observer program was launched in 1977 after the introduction of Canada’s Economic Exclusion Zone (EEZ). The program has the dual role of monitoring compliance to fisheries legislation and collecting onboard scientific data. As part of this monitoring program, fisheries observers record biological, environmental and fishing operation information, notably species identification, weight/length measurement and condition of discarded catch. Annual observer coverage of the large pelagic fleet, expressed as a percentage of sea days fished, ranges from 5 % to 18 % for domestic vessels and 100 % for foreign vessels. Over the years, data received from the observers has been of increasing quantity and quality. For example, in 2001 the observer program was marked by important changes in the deployment and reporting of observers. Firstly, length estimates and released condition of bycatch started being more consistently recorded, which was not the case prior to 2001. Secondly, to address the observation that early observer deployments were poorly distributed in time and space (Porter et al. 2000), deployments were designed to better reflect spatial and temporal distribution of the fleet.

For all species, Canadian fisheries observers have been asked to assess release condition based on injuries and movement. Individuals are coded according to the following scale: (0) Unable to determine, (1) Alive - no injury, (2) Alive - injured, (3) Dead, (4) Dead - shark bit, and (5) Moribund - near death. Also, condition of landed catch is recorded using a similar scale: (0) Unable to determine, (1) Live when landed, (2) Dead when landed, and (3) Shark bit. In principle, recording both the condition of landed and released catch is beneficial since it can differentiate between capture mortality and subsequent mortality associated with handling. However, neither measure assesses post-release mortality, which by definition, must occur after the fish has been returned to the water. Due to the fact that sea turtle bycatch in the longline fisheries is of particular conservation interest, observers have been recording additional information concerning the capture/release condition of turtles since 2001. These additional data include species, carapace length, how the sea turtle was hooked or entangled, release condition, and gear configuration. In 2011 improvements were proposed to incorporate the turtle information in to the standard observer data sheets, to collect more consistent and more detailed information, and to align terminology with U.S.A. protocols (Table 1).

It is important to note that fisheries observers have no set criteria on how to classify the condition of released individuals; therefore the coding of an individual in a category is subjective. In a detailed analysis of observer codes applied to blue sharks, Campana et al. (2009a) found that coding variability among observers sometimes overwhelmed any biological differences. Based on persistent entries of zero by some observers across trips, some observers were apparently unaware of the requirement to record the incidence of dead or injured sharks, particularly in the years 2001-2002. Injury rates were also inconsistently reported by observers, with 31% of trips reporting no injured sharks. The proportion of injured sharks reported in the remaining trips was 31.9%. Presumably, a standardized distinction between healthy and injured sharks would have reduced the variability, although data entries consistently set to zero suggest that no observations were made.

Subjectivity of observer data was further demonstrated when individual observers were tracked through time. When describing the state of sharks upon release, observers on longline boats tended to rely on code 2 more frequently than the rest, deeming the shark ‘Alive and Not Injured’ upon release. In many cases, code 2 constituted >75% of the observations made by a given observer and persisted through time and different vessel assignments (Fig. 2A and 2B). However, for three of the twelve observers monitored, the majority of observations consisted of code 3, an animal deemed ‘Alive, but Injured’ (Fig. 2C). This trend also persisted through time and applied to more than 1000 instances of shark discards from each observer. As it is highly unlikely that three observers are consistently seeing injured animals discarded, while the rest see uninjured releases, this difference is probably a consequence of the subjective nature of the coding system and the lack of detailed guidance for observers regarding what constitutes an ‘injury’.

In one case, placing an observer on a new vessel was correlated with reduced subjectivity. An observer relying primarily on code 3 spent the first part of their career assigned to the same two vessels, but following assignment to a third vessel late in July of 2002, began to rely on code 2 (Fig. 2D). This trend continued for the rest of their career, regardless of the vessel they were on. Whether this change was caused by exposure to different fishing practices on the new vessel (along with a new range of discard conditions) or by a well-timed discussion with another observer, placing observers on different vessels throughout their career may help standardize the code assignments. Subjectivity in observer scoring of condition/vitality has been modeled using random effects in the analysis of those data (Benoit et al. 2010). However, while random effects can account for some of the variability in condition scoring, they cannot account for systematic biases in the application of scoring criteria as described here.

Researchers have used data from observer programs extensively to estimate bycatch, bycatch/hooking mortality and post release mortality. For instance, Carruthers et al. (2009) estimated the odds of hooking survival of common longline bycatch using observer data. The study found that odds of survival of swordfish, porbeagle and blue sharks are two to five times higher when caught on circle hooks vs. J-hooks. An independent analysis of the observer data indicated that hook type, hook size, soak time, fishing vessel, and shark length were all significant predictors of blue shark hooking mortality, while surface water temperature and the hook type x hook size interaction were not (Campana et al. 2009a). Fishing practices associated with a particular vessel or crew contributed the most to the survival or mortality of a blue shark while on a hook.

The location and severity of the hooking injury also play a role in determining how much gear can be removed from a captured sea turtle prior to release. Turtles that have all or most of the gear removed (except deeply ingested hooks) are expected to have, on average, a higher probability of survival (Ryder et al. 2006). It should be noted that in 2003, the Nova Scotia Swordfishermen’s Association developed a Code of Conduct for Responsible Sea Turtle Handling and Mitigative Measures (Nova Scotia Swordfishermen’s Association 2003). It was added to the fleet’s Conservation Harvesting Plan in 2004 and adherence to the Code is part of license conditions. The primary purpose of the Code of Conduct is to increase post-release survival through proper gear hauling protocols, sea turtle handling guidelines, and instructions for usage of dehooking gear, but also includes recommendations to avoid captures. Dehooking and line-cutting kits to be used for the safe release of live sea turtles have been distributed to the fleet and training in using the equipment was provided. The success of this program in reducing mortality has not been evaluated. Watson et al. (2005) suggested that the use of fish bait and larger (e.g., 18/0) circle hooks may reduce loggerhead turtle bycatch and mortality. An analysis of observer data from Canadian pelagic longline fishery indicated that circle hooks >16/0 and mackerel baits were mostly used to target swordfish.

Conventional Tagging

Authors' views on the utility of conventional tagging for studies of post-release mortality differ quite substantially. Cramer (2004) in a review of data available for estimation of post-release mortality of large pelagic species concluded that low conventional tag recaptures (0.4–1.83%; Prince et al. 2002, Ortiz et al. 2003), are confounded with tag shedding, low exploitation rate, and failure to report recaptured tags so that they do not provide sufficient information to estimate post-release mortality (Bailey and Prince 1994, Jones and Prince 1998). For example, in response to reductions in quota fishermen from the West Greenland fishery for Atlantic salmon reduced the number of external tags reported, which biased tag return data (Stokesbury et al. 2009). On the other hand, the IPHC has relied extensively on this methodology to validate the condition grades described in the previous section. The different perspectives might be due to the fact that the IPHC were able to tag a large (14,872) number of individuals over three studies conducted during the 1990s, while obtaining an overall 5% recapture rate (Trumble et al. 2000). Such a reporting rate is considerably higher than that experienced in Atlantic tagging studies involving large pelagic species.

Given the problems identified by Cramer (2004), we agree that conventional tagging approaches do not offer much promise for obtaining estimates of post-release mortality.

Telemetry

Some of the earliest studies of post-release mortality using telemetry employed acoustic tagging approaches. Active acoustic tracking studies utilize individual coded acoustic tags that transmit information to a mobile ship-borne receiver. As noted by Cramer (2004) who focused on estimates of post-release mortality for billfish, acoustic tagging studies suggested relatively low post-release mortality rates for periods ranging from a few hours to a few days (e.g., sailfish, *Istiophorus albicans* (Jolley and Irby 1979); blue marlin, *Makaira nigricans* (Holland et al. 1990, Block et al. 1992); black marlin, *Makaira indica* (Pepperell and Davis 1999). However, acoustical tagging data on Atlantic white marlin are sometimes based on a very limited sample size (e.g., n = 2 tracks, Skomal and Chase 2002) and furthermore, limitations and biases of acoustic tracking study procedures may limit the accuracy of the billfish post-release mortality estimates (Pepperell and Davis 1999, Graves et al. 2002). These procedures include additional handling required to apply the acoustic tag to these animals which would be expected to increase mortality compared to mortality of animals that were only caught and released. Cramer (2004) also noted that, on the other hand, animals which are in poor shape upon capture might be selectively released without the tag, as compared to animals which were in good shape. Selection for animals that are robust would cause a positive bias in survival rates. In summary, existing acoustic data cannot be relied upon to accurately estimate the fraction of fish that survive because of small sample sizes, biases due to handling, and limited observation time.

While evaluating the usefulness of acoustic tracking studies for determining post release survival, Skomal (2007) reports that the studies are short term because of the cost in personnel and ship time and the labour intensive nature of the studies, therefore only short term survival may be examined (i.e., usually less than 12 hr). In most cases, physical trauma and physiological stress and handling of the animal were minimized, which is not representative of normal fishing practices.

Conventional satellite tags, or platform transmitter terminals (PTTs), have been widely used on all species of sea turtles. As sea turtles spend much time at the surface, compressed behavioural and environmental data from PTT-tagged animals can be regularly relayed to polar orbiting satellites, enabling not only remote collection of these data, but also geolocation of transmitters. Most applications of satellite telemetry to research on loggerhead and leatherback turtles, the two most common species of marine turtle found in Canadian waters, has focused on behaviour and habitat use (e.g., Polovina et al. 2000, Polovina et al. 2004, James et al. 2005, Hays et al. 2006, Kobayashi et al. 2008). PTTs have been integrated into studies of post-hooking mortality, but the results are not conclusive due to several confounding factors. The application of tags to 'control' animals, required to distinguish natural mortality from post-hooking mortality, has been lacking in some studies (Parker et al. 2003, Chaloupka et al. 2004). Moreover, it is often not possible to distinguish between PTT tag failure (e.g., defective tag, failure of battery or detachment of tag) and post-hooking mortality (Parker et al. 2003, Chaloupka et al. 2004, Hays et al. 2007). Chaloupka et al. (2004) concluded that PTTs cannot be used to determine post-hooking mortality unless the cause of the cessation of tag transmissions is known. Such diagnoses require the retrieval of tags that is normally not feasible. Pop-up archival transmitting (PAT) tags are an alternative to PTTs and are well-suited to survival studies of open-ocean animals. PAT tags are programmed to release, or "pop-up", from subject animals at a pre-set time, then transmit archived data continuously at the surface. Importantly, PAT tags also incorporate premature release features which can detect mortality events, such as when the tag descends beyond the depth range utilized by a live animal or remains at the surface or at depth without periodic surfacing. These additional data can be used to deduce the fate of an animal, based on certain behavioural assumptions, without necessarily recapturing the individual or retrieving the tag.

Sasso and Epperly (2007) used PATs to compare survival rates of dip-netted (control group) and pelagic longline-caught loggerhead sea turtles. Their study found no difference in survival between the lightly hooked and control turtles, and estimated annual survival rate to be 0.814 (95% CI 0.557–0.939). In 2011, Fisheries and Oceans Canada (DFO) Maritimes Region initiated a three-year project to expand on this work by increasing the sample of lightly hooked turtles and also including those that are deeply hooked (hook ingested). DFO staff plan to deploy 40-50 PAT tags to loggerhead turtles incidentally captured in the Canadian large pelagic longline fishery. This project coincides with National Marine Fisheries Service (NMFS) Southeast Fisheries Science Center plans to apply PAT tags to additional control (dip-netted) turtles. DFO and NMFS will be using the same make and model of tags, programmed with identical settings (including tracking durations and intermittent monthly reporting). An integrated break-away feature will help free turtles that become entangled in gear. Before PAT tag attachment, each turtle will be measured and also photographed to document the location of hooking. As hooking location is thought to be an important variable in the fate of loggerhead sea turtles released from pelagic longline gear (Ryder et al. 2006), inclusion of deeply hooked animals in this study would improve our estimates of post-release survival.

Compared with acoustic tag techniques, pop-up satellite archival tag (PSAT) technology provides an improved tool for evaluating post-release mortality. PSATs record environmental variables for predefined intervals, detach from an animal at a designated time, float to the surface, and transmit stored data to a satellite. These data allow analysis of post-release behaviour of tagged fish, and importantly, are not reliant upon recovery by a fishery. Early applications of this relatively new technology were promising, but the resultant mortality estimates were based on small samples and subject to similar procedural biases as described for acoustic tags, and are representative of only the gear and fishery studied (Goodyear 2002).

Examples of the limitations of the emerging technology were highlighted by Cramer's (2004) review of post-release mortality in billfishes. Nine PSAT's attached to blue marlin caught on recreational gear (Graves et al. 2002) and nine PSAT's attached to blue marlin caught on longline gear (Kerstetter et al. 2003) did not have pre-release software or an emergency release device. Without these features it was not possible to differentiate between dead fish and lost or malfunctioning tags. Therefore, the fate of the one blue marlin caught on recreational gear and two blue marlin caught on longline gear whose tags did not report is not clear. These fish may have died and sunk to a depth at which the tag was crushed, lost positive buoyancy, been eaten by sharks, or the tags could have been damaged or malfunctioned. The resulting post-release mortality estimates of 11.1% for blue marlin caught on recreational gear (Graves et al. 2002) and 22.2% for blue marlin caught on longline gear are not as reliable as estimates from more recent studies.

Pop-up archival satellite tags have had pre-release software since 2002. If the tag stays at a predetermined depth for a set amount of time (usually 4 days for fish) then the tag release mechanism is triggered, the tag floats to the surface and reports. Also, if the tag detaches from the fish prematurely and floats to the surface, it will report to the Argos satellite system. In some instances, researchers have reported what they are very sure are mortalities, usually immediately after tagging occurs (e.g., Stokesbury et al. 2004) which could indicate post-release angling-induced mortality. Interpretation of post-release mortality is virtually certain in instances where the fish has sunk to the bottom and remains immobile for pre-release time period before the tag release mechanism is activated (Campana et al. 2009b, Fig. 3). However, it is important to note that this is not always the case. For example, Figure 4 shows two tags from Canadian PSAT studies of swordfish, where the period of immobility was not observed, and the fate of those two individuals is uncertain.

While there have been significant releases of newer PSATs, the objectives of such studies have typically focussed on movements, migrations and habitat utilization (Block et al. 1998, Lutcavage et al. 1999, Stokesbury et al. 2004, Block et al. 2005, Wilson et al. 2005, Stokesbury et al. 2007). For those reasons, valuable fish are treated very carefully during tagging operations, and estimates of survival obtained from such work may not necessarily reflect the post-release mortality experienced in a fishery. However, there have been several studies designed specifically to examine post-release survival under more realistic recreational or commercial fishing conditions. Post-release mortality rates of 26% for striped marlin (*Tetrapturus audax*) and 17% for white marlin (*Tetrapturus albidus*) were reported following capture in a recreational hook and line fishery (Domeier et al. 2003, Horodysky and Graves 2005). Several studies examining post-release survival of sharks following release from a commercial pelagic longline fishery are currently underway. However, the most comprehensive study completed to date, was one examining both capture mortality and post-release survival of blue sharks caught on pelagic longlines as part of the commercial fishery (Campana et al. 2009b). In that study, estimates of capture mortality by observers (13%) and scientific staff (20%) differed significantly, presumably reflecting differences in shark examination, fishing vessels observed, and the gear that was used. However, the estimates of post-release mortality were independent of capture mortality, ranging from 0% for uninjured sharks to 33% of the injured sharks, with an overall mean of 19%. These results contrast with a total mortality rate of 5% reported for uninjured blue sharks caught as part of a research survey in the Pacific (Moyes et al. 2006). In an exchange of views in the scientific literature, Campana et al. (2009b) and Musyl et al. (2009) debated the exact source of the discrepancy, but agreed that the post-release mortality depended on fishery specific features, such as hook type, soak time, and handling of the bycatch during capture and release. A central conclusion of the two studies was that researchers must be mindful of the need to emulate fishery operations so that the estimates of survival obtained using PSATs are representative of those experienced in the fishery. More generally, there may also be a strong potential for a cluster effect in the estimated mortality obtained from PSAT studies. If fish are tagged from a small number of fishing sets (possibly even during a single trip), estimated mortality may not be representative of discards in the broader fishery.

A summary of apparent survival rates as indicated from a literature review of telemetry studies is presented in Table 5 for species of interest to this review. The minimum post-release survival of the studies reviewed is approximately 80%.

Given the high cost of conventional PSATs (currently around $3500 per unit, plus satellite transmission charges which vary with deployment duration), there is understandable interest in the minimum sample size that will still permit robust inferences of post-release mortality. Goodyear (2002) concluded that for black marlin (*Makaira indica*) 100 fish would need to be tagged, although Stokesbury et al. (2011) recently suggested that a minimum of 50 bluefin tuna would need to be tagged during a simulated catch and release recreational fishery to achieve an estimate of post-release mortality with acceptable precision. Those same authors later obtained an estimate of post-release mortality (within 30 days of tagging) of 3.4% (95% C.I. = 0.8%<u<12.6%) based on 59 tags (Stokesbury et al. 2011), suggesting that lower tag numbers would have resulted in estimates of ±10%. Supporting that view was a study of post-release mortality in blue sharks released alive from commercial longliners, which produced an estimate of 19% (95% C.I. = 10%<u<29%) based on 40 tags (Campana et al. 2009a). Based on the above results, a minimum of 40 tag releases would be required to produce mortality estimate within 10% of actual values, with more tags producing better precision. Note that tag number is not directly proportional to precision; halving the uncertainty to 5% from 10% would require 4 times as many tags.

Tag manufacturers have started to realize that there is a demand for mission-specific electronic tags to determine post-release mortality, and that unit cost has been a barrier to more broad application of the methodology. Inexpensive prototype units ($1000-$2000 per unit) have been developed and tested by both Canadian and U.S.A. manufacturers, with encouraging results. Figure 5 illustrates results of a blind test of a prototype design, where caged Atlantic salmon were sacrificed following a schedule which was unknown to the tag manufacturer. Using the Argos transmitted or archived information, the manufacturer was able to accurately determine the hour of death, although the sample size was small (n=10). There is also a general trend towards smaller satellite tags, which should make the telemetry option for determining post-release mortality feasible for a broader range of species.

Hoolihan et al. (2011) has produced a review of post-release behaviour modification following application of PSATS to various species of large pelagic fish. Those authors evaluated using empirical eigenfunction analysis to detect changes in vertical movement patterns recorded by 183 PSATs. While those authors did not specifically address post-release mortality, they did comment that periods of irregular post-release behaviour lasted from 3 to 60 d (mean = 15.8, s.d. = 10.4), which was thought to reflect a stress response. In a study of post-release mortality of blue sharks, reported significantly longer periods of irregular post-release behaviour in injured sharks compared to those that were uninjured at release. The same study noted that 95% of the post-release mortality occurred within 11 days of release. These results may give some insight into the appropriate periods for deployments of PSATs for studying post-release mortality. It was also noteworthy that the three shark species of interest to this review exhibited a higher incidence of unusual vertical migration behaviour compared with swordfish and bluefin tuna (Fig. 6).

In common with other approaches, PSAT methods could be criticized for the absence of controls, thereby making it difficult to quantify mortality that could be attributed to the tagging event, and the impacts of the fish carrying the PSAT tag after release. However, some workers have attempted to characterize mortality associated with capture by different gear types, with the assumption that capture by certain gear could be considered controls. For example, Sasso and Epperly (2007) compared survival rates of dip-netted and longline-caught turtles, and considered the dip-netted individuals to be the control group. They found no difference in survival between the two groups, and the estimated annual survival rate was 0.814.

Biochemical Methods

Biochemical methods consist of correlating physical or physiological response indicators with mortality. Fish capture results in some physical and/or physiological trauma, regardless of gear employed, and measures of such trauma or the accompanying physiological response can be used as post release mortality indicators (Farmer et al. 1998, Rose 1999, Pranovi et al. 2001). For fish, physiological response indicators have commonly included blood concentrations of cortisol, lactate, glucose, chloride, sodium, potassium and haematocrit, while physical indicators include scale loss, bruising and wounds (Beamish 1966, Main and Sangster 1988, Oddsson et al. 1994, Turunen et al. 1994, Kaiser and Spencer 1995, Suuronen et al. 1996a, 1996b, 1996c, Broadhurst et al. 1997, Olla et al. 1997, Soldal and Engas 1997, Olla et al. 1998, Broadhurst et al. 1999, DeAlteris and La Valley 1999, Davis and Olla 2001, Parker et al. 2003). Similar variables have been collected for other organisms, for instance L-lactate, ammonia, D-glucose, adenylic energetic charge, skeletal or shell damage, arm or limb loss and body mass changes (Meyer et al. 1981, Bergmann and Moore 2001a, 2001b, Ramsay et al. 2001, Broadhurst et al. 2002, Maguire et al. 2002, Harris and Ulmestrand 2004, Macbeth et al. 2006). Detailed discussion of merits of various physical and physiological indicators is beyond the scope of this review, although it is apparent that their benefit in terms of providing a tractable relationship with mortality has varied considerably. For example, Beamish (1966) demonstrated a positive correlation between peak concentrations of blood lactic acid (fatigue measure) in haddock and their discard mortality. Conversely, Oddsson et al. (1994) and Davies et al. (2009) failed to demonstrate any conclusive relationships linking several stress indicators and discard mortalities of Pacific halibut and sablefish, respectively.

Application of biochemical approach to large pelagic fishes has focused on quantifying changes in blood constituents (Manire et al. 2001, Skomal and Chase 2002, Skomal 2007). For instance, Moyes et al. (2006) used blood samples from blue sharks and identified five variables differentiating moribund sharks and survivors that were used to develop logistic regression models to predict long term survival. Unfortunately, this study did not sample blood from any sharks which subsequently died, thus limiting the predictive value of the model. Musyl et al. (2009) suggested that biochemical methods may be an advantageous alternative to the use of costly telemetry studies, as it would optimize experimental design by reducing experimental bias (preferential tagging of individuals) and increasing sample size due to lower cost. Once an appropriate calibration between the biochemical method and objectively-determined post-release mortality is completed, the biochemical method may indeed have value. However, there are key issues associated with their use on large pelagic fishes. Determination of a baseline of physiological indicators prior to gear encounters is difficult, as individuals are unlikely to maintain a stress-free state during the collection of biochemical samples (Post et al. 1997, Skomal 2007). In this respect, biochemical and physical studies are similar, since both could provide a cost-effective means of estimating post-release mortality once their predictive accuracy has been tested. However, to derive post release mortality estimates, it is essential to conduct quantitative studies to determine the fate of released fish. Therefore, in the absence of telemetry, biochemical methods yield little information on the fate of released fish (Skomal 2007) and thus are not recommended for use in large pelagic fishes until the appropriate baseline levels and linkages to actual mortality have been developed.

CONCLUSION AND SUGGESTED GUIDELINES

Pollock and Pine (2007) considered that either containment studies or telemetry methods are suitable methods to assess post-release mortality. They argued that containment studies are relatively simple to design and implement, whereas telemetry studies are more complex and likely more expensive. The trade-off is that containment studies take place in an unnatural setting while telemetry studies allow the animal to be returned to the wild and provide additional information common to telemetry studies such as movement and habitat use. This is a major plus for telemetry studies; however, care must be taken to minimise the effects of the telemetry tag on animal behaviour and ultimately survival, and that the experimental protocol mimics the fishery to the greatest extent possible.

We consider that for the relatively large species of interest in the Canadian pelagic longline fishery, confinement approaches have little promise. Rather, the most effective methods are likely a combination of appropriate release condition codes, standardized across observers that are validated using telemetry. Development of standards that help observers categorize release condition is also strongly recommended, and could include photographs as part of a field manual.

Recognizing that studies of this nature will be expensive, it would be helpful to prioritize the requirements for the new information based on considerations of the quantity of by-caught species, the status of the resource (if known), and the likelihood that unilateral Canadian management actions will result in an improvement of resource status (Table 6).

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Table 1. Proposed capture and release conditions of turtles recorded by fisheries observers in the Canadian pelagic longline fishery.

Codes	Type of Interaction (Hook Location)
10	Entangled
20	Beak External, Unknown
21	Beak External, Lower Jaw
22	Beak External, Upper Jaw
30	Unknown Internal
31	Mouth Internal Unknown
32	Mouth Internal, glottis
33	Mouth Internal side
34	Mouth Internal, roof of mouth
35	Mouth Internal , tongue
36	Beak Internal, Unknown
37	Beak Internal, lower jaw
38	Beak Internal , upper jaw
41	Swallowed (esophagus; hook visible to insertion point)
42	Swallowed (esophagus; hook partially visible)
43	Swallowed (esophagus; hook not visible)
44	Swallowed (esophagus; hook visibility unknown)
61	Unknown External
62	Head
63	Neck
64	Carapace
65	Plastron
70	Rear Flipper, Groin, Tail (exact location unknown)
71	Rear Flipper
72	Groin
73	Tail
90	Front Flipper or Shoulder or Armpit (exact location unknown)
91	Front Flipper
92	Shoulder
93	Armpit

Code	Turtle release condition
1	all gear removed
2	gangion cut next to hook
3	gangion cut away from hook
4	no gear removed
6	unable to determine (UTD)

Table 2. Four level vitality code used by fisheries observers in the study of Benoît et al. (2010) for groundfish in the Gulf of St. Lawrence.

Vitality	Code	Description
Excellent	1	Vigorous body movement; no or minor[a] external injuries only
Good/fair	2	Weak body movement; responds to touching/proddingl; minor[a] external injuries
Poor	3	No body movement but fish can move operculum; minor[a] or major[b] external injuries
Moribund	4	No body or opercular movements (no response to touching or prodding)

[a] Minor injuries were defined as ‘minor bleeding, or minor tear of mouthparts or operculum (≤10% of diameter), or moderate loss of scales (i.e. bare patch)’.
[b] Major injuries were defined as ‘major bleeding, or major tearing of the mouth-parts or operculum, or everted stomach, or bloated swim bladder’.

Table 3. Percentage of captive fish that survived at least 48 h, by condition code, as determined by Benoît et al. (2010) for groundfish in the Gulf of St. Lawrence.

	Vitality code			
Species	1	2	3	4
American plaice	88.1 (109)	64.8 (71)	53.8 (52)	3.9 (642)
Atlantic cod	65.1 (43)	39.4 (66)	14.8 (54)	1.9 (483)
Atlantic halibut	100 (3)	-	50.0 (5)	-
Skates	100 (56)	100 (31)	62.5 (24)	42.1 (38)
Sculpins	100 (19)	100 (20)	83.3 (12)	55.6 (9)
White hake	100 (2)	66.7 (3)	50.0 (4)	-
Winter flounder	100 (19)	92.0 (25)	63.2 (19)	19.1 (47)
Witch flounder	-	75.0 (8)	50.0 (14)	-

Table 4. Longline injury codes currently used by the International Pacific Halibut Commission (provided courtesy of G. Williams, IPHC).

Key to Longline Injury Codes for Pacific Halibut

Codes : 1 = Minor, 2 = Moderate, 3 = Severe, 4 = Dead/Sand Fleas/Bleeding, 9 = Unknown

1a. Fish is alive .... Go to 2a

1b. Fish is dead when brought to the surface on the gear .... Code DEAD
Fish is in rigor and lifeless, even if no apparent injuries. Gills appear completely devoid of blood (light pink or white in color).

2a. Body shows no signs of marine mammal predation .... Go to 3a
Fish's body is intact. Flesh may be torn, but no missing tissue.

2b. Body is missing pieces of flesh .... Code DEAD
Pieces of tissue are missing from predation by marine mammals. Missing pieces are typical of bites from sea lions or other large marine mammals.

3a. No penetration of the body or head by sand fleas .... Go to 4a
Membranes surrounding eyes and anus are intact, without any holes from sand fleas. A few sand fleas may be seen on body and can be wiped off with your hand. Typically, no penetration occurs when only a few (e.g., <10) sand fleas are found on the body.

3b. Sand fleas have penetrated the body via the eyes, fins, or anus .... Code DEAD
Membrane surrounding eye may be partially or completely missing. Dorsal and/or anal fin membranes may be eaten away, leaving fin rays exposed. Skin on the body is separated from tissue where sand fleas have eaten.

4a. No wounds of any kind to abdominal organs. Abdominal wall not punctured .... Go to 5a

4b. Abdominal organs are damaged, possibly by a gaff .... Code DEAD
Abdominal cavity wall is punctured or torn. Viscera are visible and exposed, and may be protruding.

5a. Fish is not bleeding from gills (but may be bleeding from elsewhere) .... Go to 6a

5b. Fish is bleeding from gills .... Code DEAD
Bleeding is occurring from a torn or severed gill arch.

6a. Fish is not bleeding at all, or bleeding is minor to moderate (not from gills) .... Go to 7a
Blood may be seen around mouth and/or jaw. Blood may be oozing continuously, or bleeding may be continuing very slowly a few drops at a time, or bleeding may have stopped.

6b. Bleeding is severe .... Code DEAD
Blood from any source is flowing freely and continuously in large quantity.

7a. Injuries to head and/or jaw are minor to moderate, but no structures are missing .... Go to 8a

7b. Major injuries to head and jaw, resulting in missing pieces .... Code SEVERE
Side of the head, possibly including the jaw, has been torn loose and missing from the fish, and/or lower jaw has been torn away and is missing.

8a. Wounds to the head (forward of preopercle and above cheek and jaw) are only surface scratches on the skin .... Go to 9a

8b. Skin on head (forward of preopercle) is ripped and torn deeply .... Code SEVERE
Internal organs are likely exposed.

9a. Eye or eye socket is not punctured .... Go to 10a

9b. Eye or eye socket is punctured .... Code MODERATE

10a. No wounds to the body are evident .... Go to 11a

10b. Wounds in body consist of puncture holes in skin, with possibly a flesh tear .... Code MODERATE

11a. Lower jaw is significantly damaged .... Code MODERATE
Lower jaw may be broken into 2 pieces at the snout, but each is still attached at the base of the jaw. Jaw may be torn on one side or the other, possibly extending through the cheek.

11b. Damage to lower jaw, if any, is slight .... Code MINOR
Injuries include the hook entrance/exit hole around the jaw or in the cheek, or a tear in the cheek. A piece of the lip may be torn and hanging from the jaw. If gangion was cut, the hook and some length of residual gangion may be hanging from the mouth.

gbw/iphc

September 12, 2000

Table 5. Post release survival estimate for six of the seven bycatch species from studies using satellite tagging technology (provided courtesy of M. Stokesbury, Acadia University). No information is available for short fin mako. Note that a variety of methods of capture were used.

Species	Reference	N	Length (cm)	Est. Weight (Kg)	Hooking Method	Tagging Procedure	Mort	Non-report	Min Sur (%)
Bluefin Tuna	Block et al. 1998	37	-	96 - 181	-	OD	0	2	95
	Lutcavage et al. 1999	20	4: 190-200 8: 201-225 8: 226-263	-	-	OTS	1	-	85
	Lutcavage et al. 2000	11	-	Mean = 229 SD = 94	Harpoon	OTS	0	0	100[d]
	Brill et al. 2001	5	SFL mean = 90 SD = 13	Mean = 12 SD = 5	Troll	OD	0	0	100[d]
	Stokesbury et al. 2004	9	CFL mean = 243 SD = 17		Purse seine	OTS	0	1	89
	Stokesbury et al. 2004	26	CFL mean = 232 SD = 32		Troll	OD	1	2	85
	Wilson et al. 2005	3	-	Mean = 147 SD = 49	Trolling	OTS	0	0	100
	Wilson et al. 2005	65[a]	-	Mean = 161 SD = 45	Purse seine	OTS	1	8	86
	Stokesbury et al. 2007	6	CFL mean = 232 SD = 16	-	Troll	OD	0	3	50
	Block et al. 2005	239[b]	CFL mean = 207 SD = <20	-	Trolling	OD	0	21	87
	Block et al. 2008	15[c]	CFL mean = 262 SD = 18	-	Drifting	OD	1	4	66
	Total	*436*	-	-	-	-	*6*	*41*	*89%*
Swordfish	Sedberry and Loefer 2001	29	-	-	Longline	OTS	9[e]	6	69
	Canese et al. 2008	19	-	14 - 45	Harpoon	OTS	2	6	90
	Total	*48*	-	-	-	-	*11*	*12*	*79.5%*
Porbeagle	Pade et al. 2009	4	160 - 180	-	Rod and Line	-	0	0	100
	Total	*4*	-	-		-	*0*	*0*	*100%*
Blue Shark	Moyes et al. 2006	11	-	-	Longline	OD	0	11	95
	Campana et al. 2009b	40	≈ 200	-	Longline	OD	9	3	65

Species	Reference	N	Length (cm)	Est. Weight (Kg)	Hooking Method	Tagging Procedure	Mort	Non-report	Min Sur (%)
	Total	*63*	-	-	-	-	*9*	*15*	*81.2%*
Loggerhead Turtle	Hays et al. 2003	50	-	-	-	-	6	0	88
	Sasso and Epperly 2007	39	43 - 60	-	Longline	OD	3	4	81
	Polovina et al. 2004	26	-	-	-	-	0	0	100
	Total	*115*	-	-	-	-	*9*	*0*	*90%*
Leatherback Turtle	Hays et al. 2006	9	-	-	Tagged on beach	-	0	0	100
	Benson et al. 2007	19	-	-	Tagged on beach	-	0	2	90
	Total	*28*	-	-	-	-	*0*	*2*	*95%*

OD = On-Deck, OTS = Over the side

[a]Fish that were tagged and tags did not report (8), or that died (1) were not included in the weight estimates.
[b]Data from Stokesbury et al. 2004 has been removed from Data reported in Block et al. 2005. PAT data from Block et al. 2001 is included.
[c]Lengths only for the 11 fish whose tags popped up.
[d] Short term deployments
[e] Pre-mature release not necessarily mortalities.
[f]Deep hooked (0.34) and light hooked (0.08) failure rate. Small sample size and high number of tag failure.

Table 6. Prioritization of the need for studies of post-release mortality for the seven species examined. The assignment of priority was subjective, but considered the availability of information from completed studies, stock status, and the scale of the discards in relation to the catch.

Bycaught Species and Stock	Priority	Comments
West Atlantic Bluefin tuna	Medium. Study* completed in southern Gulf of St. Lawrence	Relatively small bycatch, but stock status is controversial. Results from GSL apply to that recreational fishery only.
North Atlantic Swordfish	Low	Rebuilt stock, discards relatively small compared with landings. Existing pop-up satellite archival tags (PSAT) results* may give some insight, although not designed for post release mortality estimates specifically.
Shortfin Mako	Medium	Discards are comparable to landings in some fisheries, so post-release mortalities could influence view of stock status.
Blue shark	Low, work already completed	See Campana (2009)*. Could change priority if there is a need to resolve differences with other blue shark studies.
Porbeagle shark	High	Discards exceed landings; PSAT-based study* to commence in 2013
Loggerhead sea turtle	High	PSAT-based study* to commence this field season.
Leatherback sea turtle	Low	2006 Leatherback Sea Turtle Recovery Plan suggests that mortalities are low. Recovery strategy scheduled for update in early 2012, COSEWIC reassessment scheduled for April 2012.

* Funded in total or in part through the International Governance Strategy.

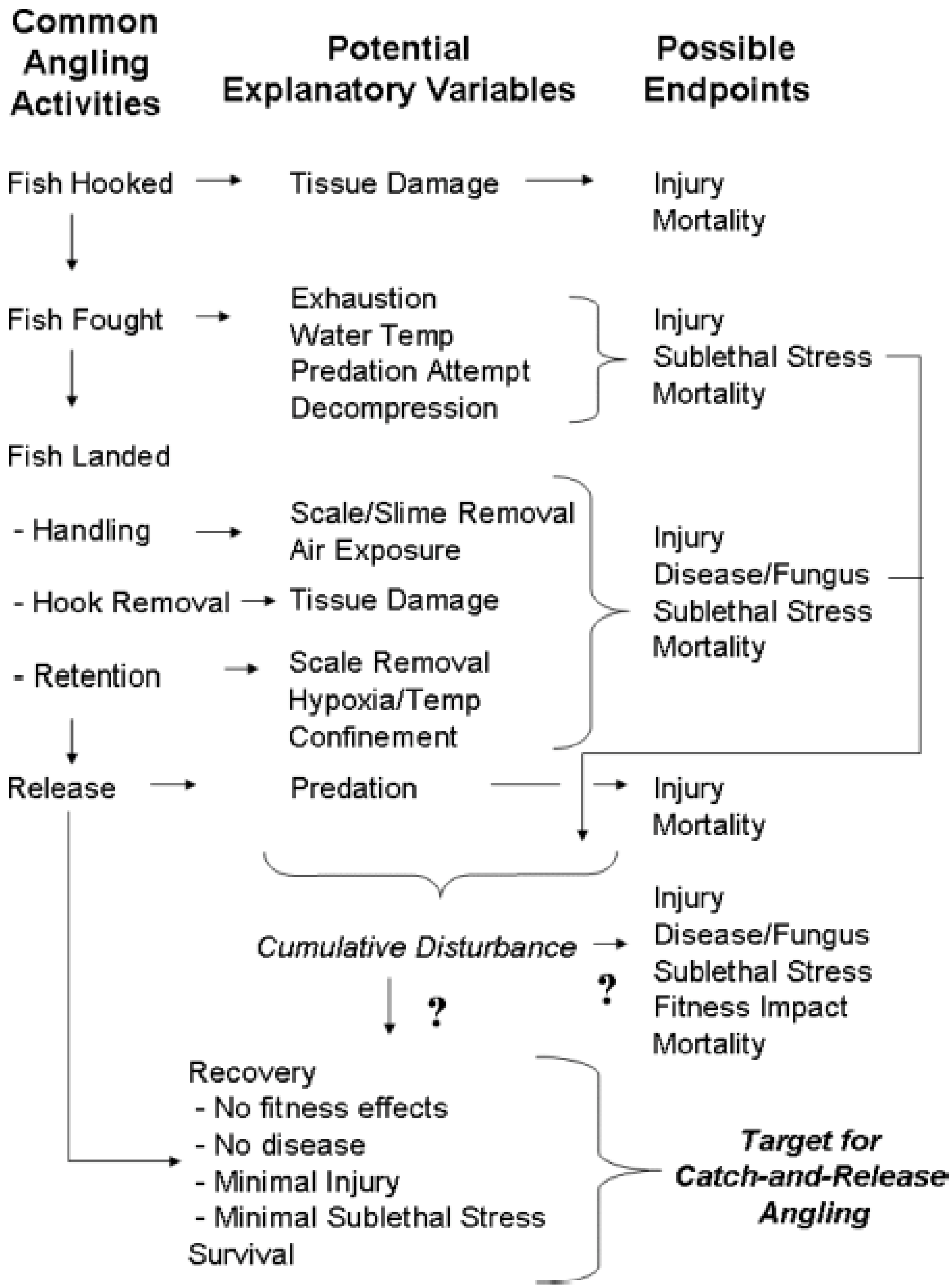

Figure 1. Simplified schematic of some of the primary factors that affect catch-and-release endpoints from a biological perspective. This schematic is from a review of freshwater recreational catch and release fisheries (Arlinghaus et al. 2007) but is applicable for most fisheries involving hook and lines. Question marks indicate gaps of knowledge.

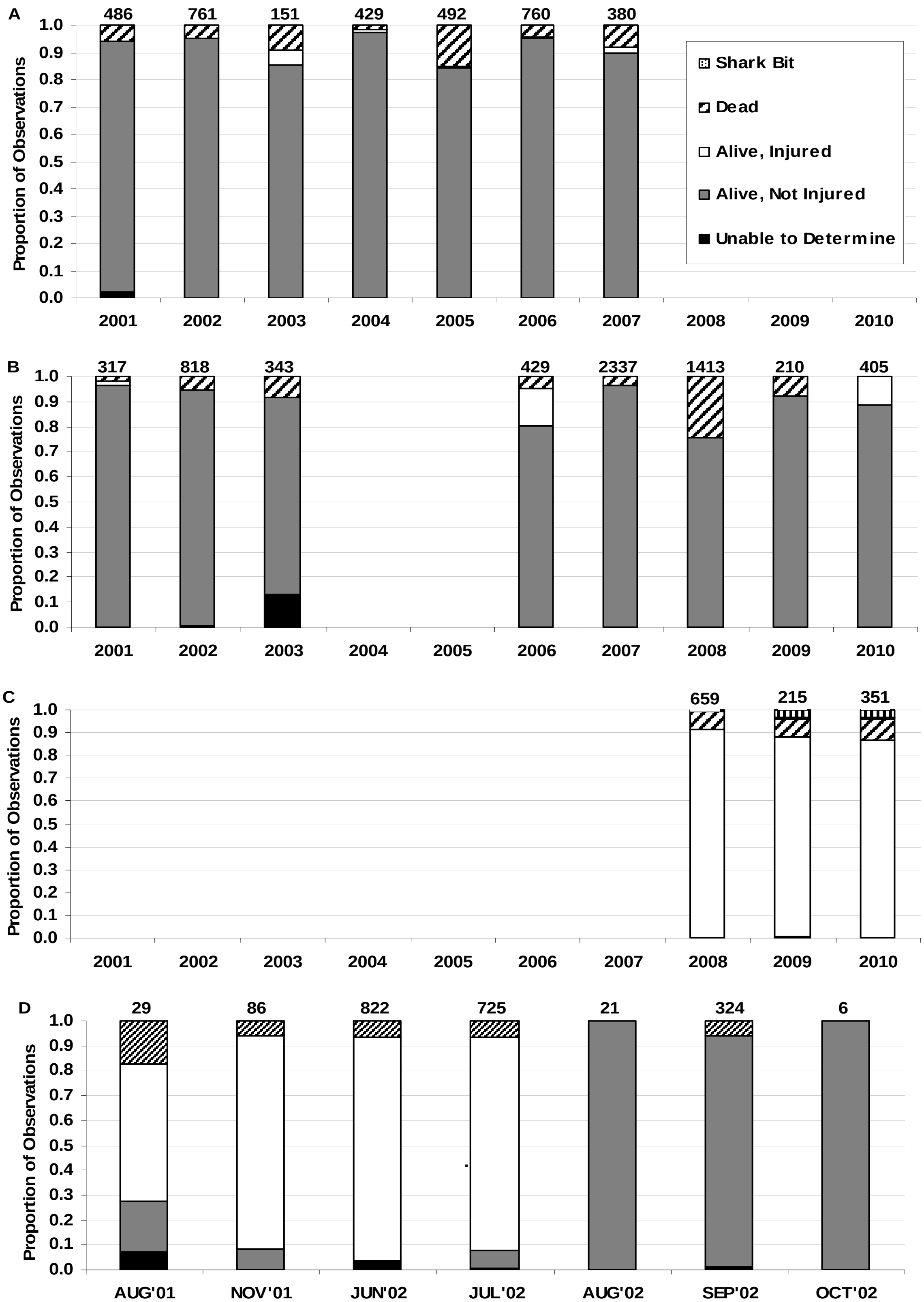

Figure 2. Annual (A - C) and monthly (D) use of 'Release Type' codes by four select observers for three shark species (porbeagle shark, shortfin mako shark and blue shark) in the pelagic LL fishery. Number above each bar indicates total number of observations made by observer in a given year or month (N).

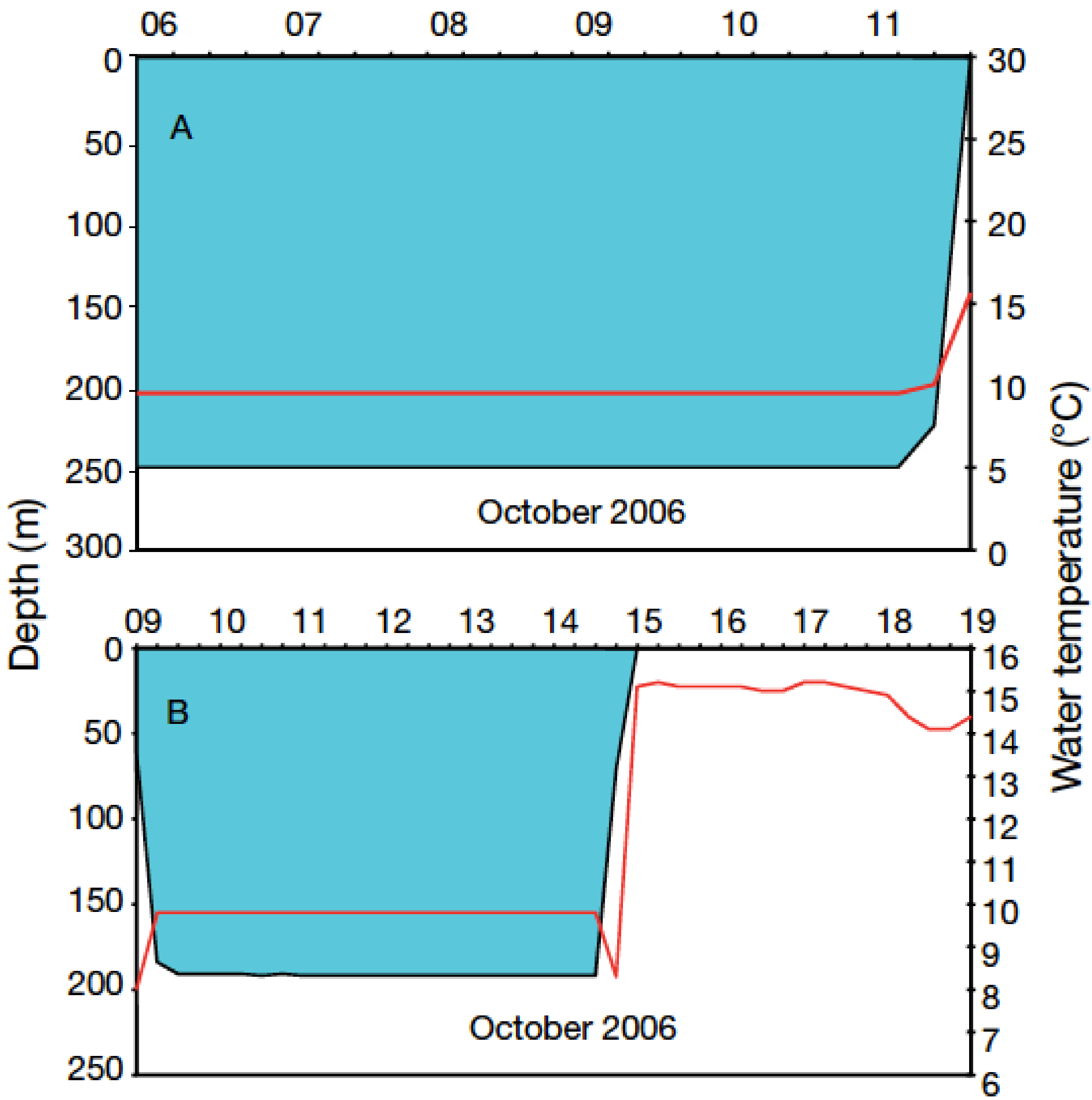

Figure 3. Examples of blue shark mortalities indicated from PSAT studies (from Campana et al. 2009b). Mean depth weighted by time-at-depth is shown in blue; time-weighted temperature indicated by red line. (A) Shark known to be dead at time of discarding. (B) Shark which appeared to have died shortly after discard.

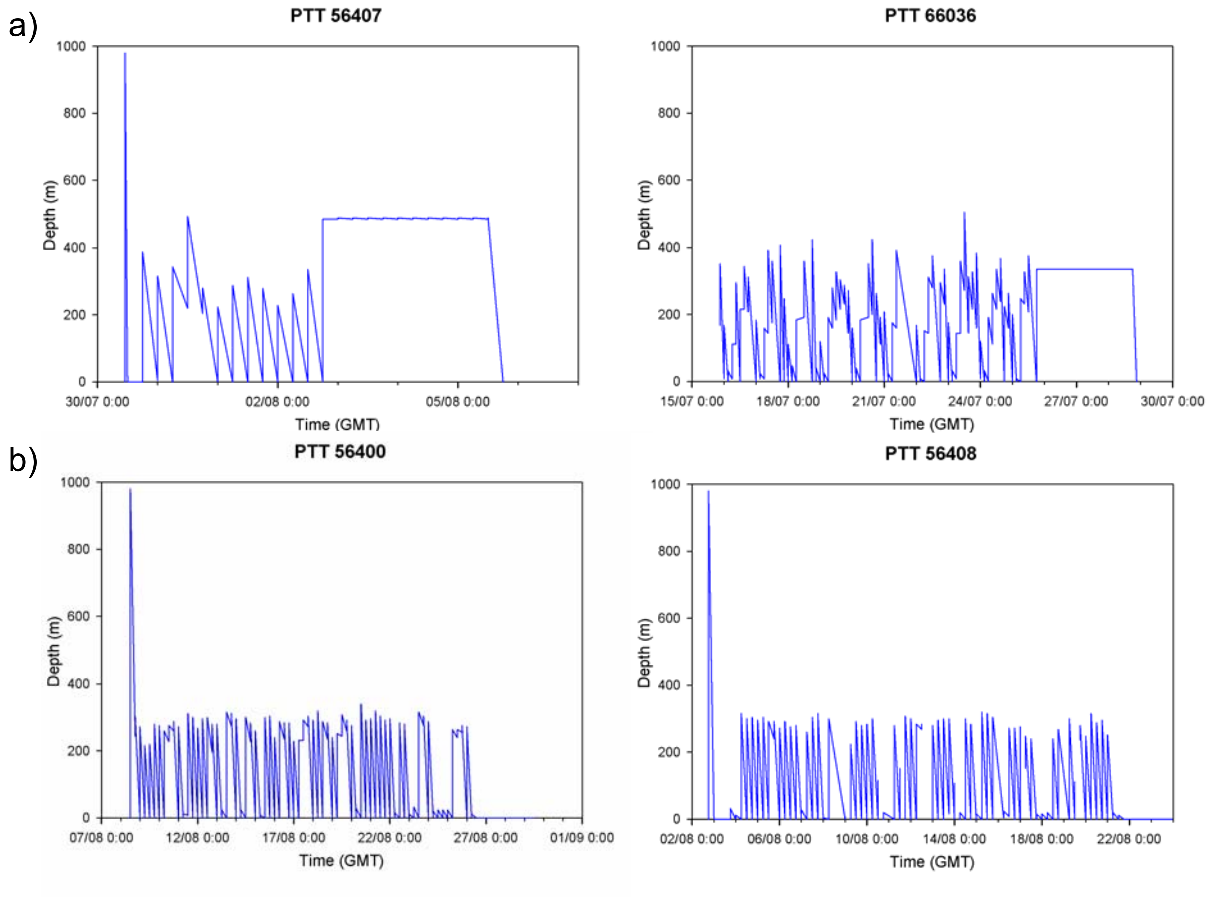

Figure 4. Depth profile of swordfish attached with pop-up satellite tags, where the premature release mechanism was activated. a) example of Swordfish where post-release mortality is virtually certain, b) example of Swordfish where interpretation of post-release mortality is ambiguous.

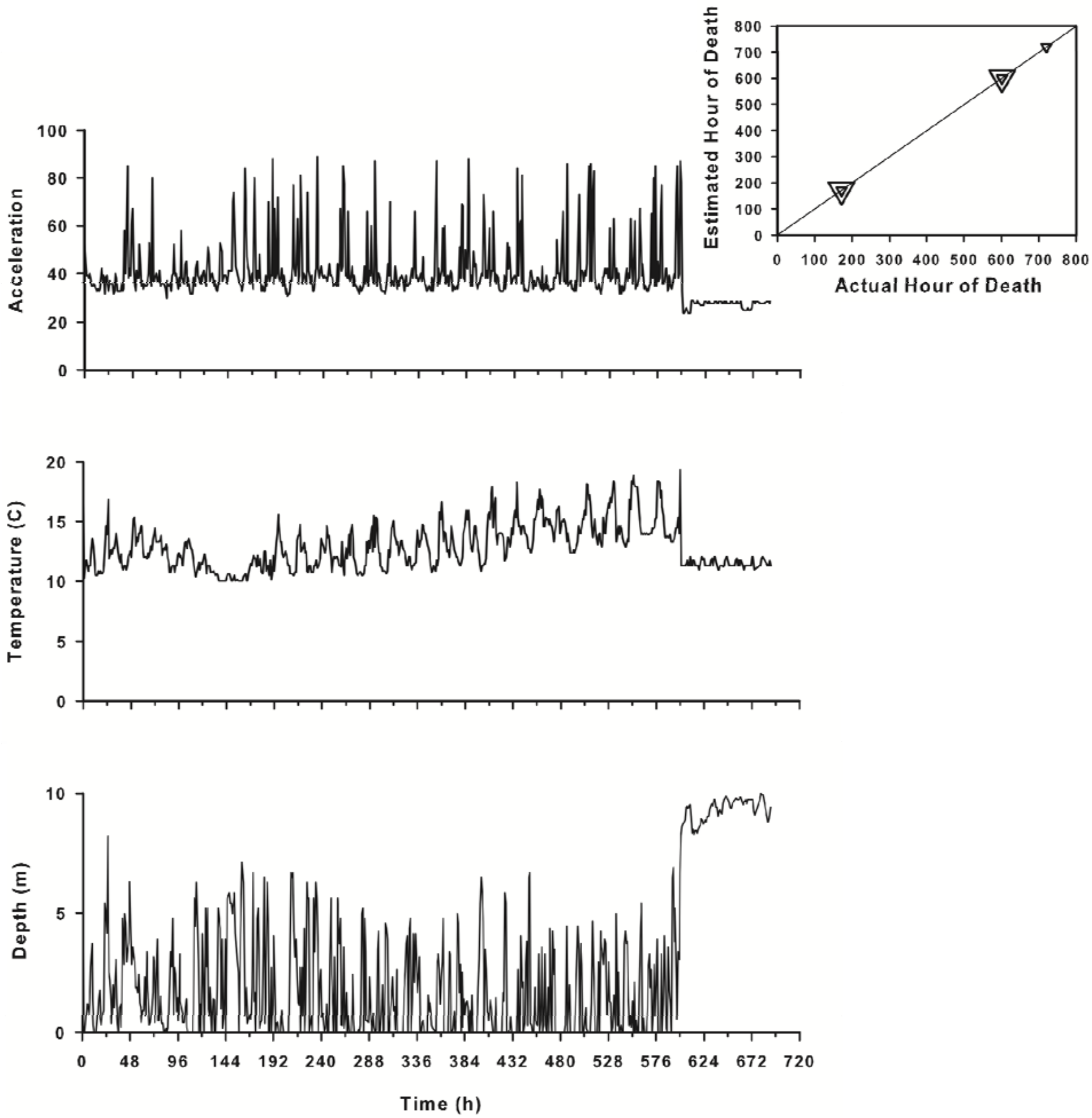

Figure 5. Time-depth, time-temperature and time-acceleration profiles from a prototype satellite archival tag attached to an individual Atlantic salmon killed at 601 hour. The fish were held in enclosures and were killed followed a schedule which was not supplied to the tag manufacturer. The estimates of time of death provided by the manufacturer compared with actual time of depth is shown in the plot on the top right of the figure.

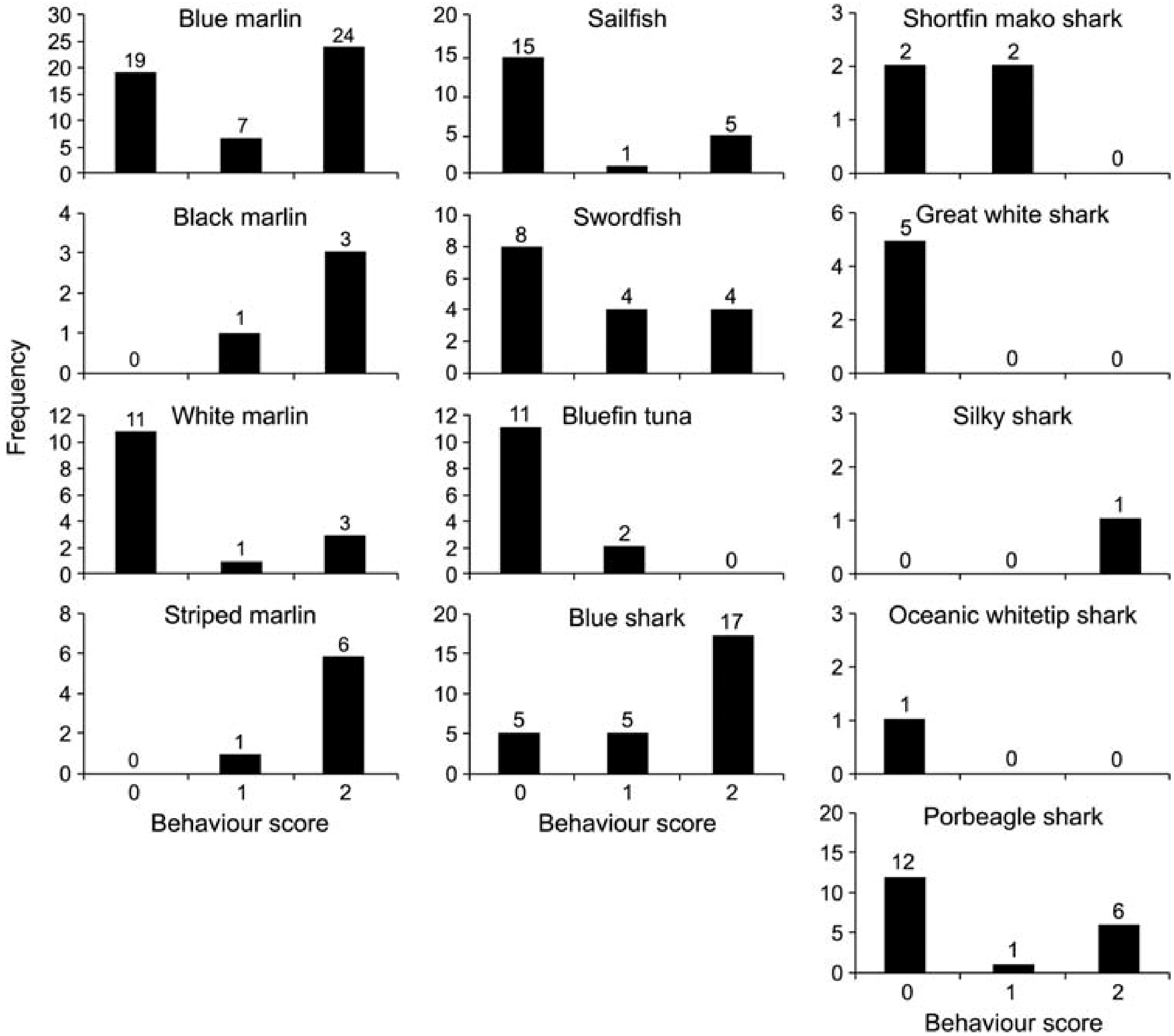

Figure 6. Frequency histograms for the occurrence of irregular post-release behaviour indicated by PSAT records from of 183 large pelagic fish from Hoolihan et al. (2011). Irregular behaviour was scored as follows: 0, no apparent change in behaviour; 1, possible presence of irregular behaviour; 2, irregular behaviour apparent.