ICES Guidelines on Methods for Estimating Discard Survival

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9 Captive observations

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Captive observation is a common technique, where discarded animals are transferred into containment facilities (e.g. tanks or underwater cages) after experiencing *in situ* representative fishing conditions (i.e. capture, handling, and release). However, the experimental subjects are not actually discarded, but are retained in captivity for a period of time to monitor their vitality and survival.

This approach facilitates the monitoring of the experimental subjects, and allows both dead and surviving animals to be sampled and assessed for injuries, physiological status, and vitality. However, it also introduces some potential limitations with respect to the applicability of the survival estimates. Firstly, holding wild animals, unaccustomed to captivity, can induce stress (Snyder, 1975; Portz *et al.*, 2006), and thereby can potentially induce captivity-related mortality in addition to the treatment effect. Controls can be used to determine whether method-induced mortality has occurred (Section 6). Also, most examples of this technique will isolate the captive population from their natural predators, so it will not account for any predation on discard survival (e.g. Raby *et al.*, 2013).

In this section, the implications for holding and monitoring animals in the field or laboratory are discussed, as well as information on designing and the practical considerations of conducting captive observation assessments.

9.1 Field vs. laboratory assessments

Captive observation can be conducted either in the field, using tanks or cages, or in the laboratory under controlled conditions. To provide estimates of discard survival which are relevant to real fishing operations, the experimental subjects must experience conditions during capture, handling, and release that are representative of the fishing operation under study. This is best achieved by sourcing the subjects for monitoring on board commercial fishing vessels, and conducting the monitoring either in holding facilitites in the field or in a laboratory. Laboratory assessments, which do not contain any element of fieldwork, can be appropriate when researchers want to investigate the isolated effects of specific variables on discard survival. In these cases, the aim is not to generate a disard survival estimate that is representative of a fishery.

9.1.1 Field assessments

Captive observation field assessments can be defined as investigations that estimate discard survival using experimental subjects collected under realistic and representative fishing conditions (e.g. Broadhurst and Uhlmann, 2007; Enever *et al.*, 2008; Raby *et al.*, 2013; Revill *et al.*, 2013; Depestele *et al.*, 2014). The subjects are then transferred to containment facilities (e.g. tanks or cages) that are either field- or laboratory-based. Frequent reporting on this technique illustrates the feasibility and acceptable costs associated with this approach. The primary advantage of this technique is that the animals under study are collected from authentic fishing conditions and have, therefore, been exposed to realistic and combined stressors associated with

the capture and discarding processes. For this reason, the results from studies conducted in this manner are likely to be trusted by the fishing industry.

A key consideration with captive observation is that it does not account for predation, and therefore potentially overestimates discard survival levels. This factor must be made explicit when presenting the results (Raby *et al*., 2013). Captivity may also exclude stressors that would otherwise be experienced by discarded fish. Therefore, survival may also be overestimated if subjects survive better in the containment facilities than if released. However, in general, the additional stressors associated with being contained are considered to have a stronger effect on subjects than any possible benefits, i.e. the method is more likely to induce mortality than to increase survival (Portz *et al*., 2006).

Captivity in tanks or cages, and transfer of organisms from the fishing operations to holding tanks, can induce additional handling and captivity stress and, therefore, requires careful use of appropriate controls. When captivity stress is observed, survival estimates observed in the treatment subjects may underestimate the true value. In addition, captive observation studies can be expensive and consequently suffer from low levels of replication, which can mean that the results may not be representative of the management unit, and that the statistical power of the data will be reduced. Integration with vitality assessments (Section 8) and tagging/biotelemetry assessments (Section 10) can be used to substantially increase the utility of the discard estimates derived from captive observation (see Section 4 for further discussion).

9.1.2 Laboratory assessments

Captive observation laboratory-based assessments can be defined as those used to investigate isolated variables and their effects on the behaviour, physiology, and survival of subjects under controlled conditions. Although conditions in laboratory experiments can attempt to emulate fishing practices, representative stressors are not usually obtainable. Therefore, laboratory-based experiments are not usually suitable for generating discard survival estimates *per se*.

Laboratory assessments do permit detailed studies into the mechanisms of mortality and injuries using untreated controls as a baseline. Such studies also allow the researcher to isolate factors, singularly or in combination, and estimate their relative importance for fish vitality and their effect on survival. Laboratory assessments also offer the opportunity to undertake post-mortems and physiological investigations, and allow many replicates, thus providing greater statistical power. With increasing focus on animal welfare, laboratory assessments also allow smaller numbers of animals to be used in experiments.

As stated above, the controlled conditions of the laboratory cannot replicate commercial fishing conditions and the interaction of stressors experienced by fish. The subjects undergoing treatment may also not be representative of commercially caught fish (Section 9.2.1). Subjects kept in captivity for longer periods can become acclimatized to captivity and potentially behave differently than "wild" specimens. Finally, it is essential to keep the experimental subjects under conditions as close as possible to those in nature.

9.2 Designing a captive observation assessment

The design of an effective captive observation assessment depends on four key elements:

i) obtaining a representative subject population;

ii) transfer into captivity;

iii) containment in appropriate conditions; and

iv) monitoring.

At each stage of the study, it is important to minimize the effects of captivity on the experimental subjects. Captivity should not be detrimental to the vitality of the subjects. This is achieved by ensuring that the holding conditions and containment facilities correspond to the subject's biological and behavioural needs as far as possible. In addition, controls can be used to determine whether there is any method-induced mortality (Section 6). This section only briefly reviews the most pertinent aspects, but there are useful and detailed guidelines available for keeping aquatic animals in captivity (e.g. Nickum *et al*., 2004; Portz *et al*., 2006; Jacklin and Combes, 2007).

9.2.1 Obtaining a representative subject population

Experimental subjects should have been exposed in a controlled or measurable way to suitable stressors that are representative of normal fishing conditions, based on the key influencing factors (technical, environmental, biological; sections 2 and 7). Test subjects will usually have been caught in a standard fishing operation, handled according to normal fishing practices, and, at the point when they would be released to the water, transferred into captivity.

9.2.2 Transfer into captivity

Following treatment, the experimental subjects are transferred to a containment facility (e.g. tank or sea cage) for monitoring. Ideally, this transfer should be representative of the conditions the discarded fish would normally experience during release. This includes both handling protocols, and anticipated changes in environmental conditions between the surface and the habitat to which they would normally return (e.g. temperature, depth, and light intensity). For example, if fish are released via a chute or pipe from the side of the vessel, this could be effectively simulated by fitting the receiving sea cage to the outlet and then sinking the filled cage to the seabed. Where this is not practical, the effects of the transfer should be controlled to minimize any associated stress and injury (e.g. minimizing air exposure).

In some instances, the transfer will involve handling individual fish. This may provide an opportunity to conduct a vitality assessment, with commercial handling times and conditions, still within normal ranges (Section 8). However, sometimes the transfer may involve many fish at the same time (e.g. when slipping small pelagic fish from purse-seines; Huse and Vold, 2010; Tenningen *et al*., 2012). In this case a vitality assessment can be conducted for a subsample of fish at different stages of the transfer, and any potential influential stressors should be monitored (Section 7).

9.2.3 Containment facilities

Conditions in the containment facilities should ideally correspond to the biological and behavioural needs of the species under investigation (e.g. Breen, 2004; Nickum *et al*., 2004; Broadhurst *et al*., 2006; Jacklin and Combes, 2007). These needs will often be species-specific. As examples: flatfish require a non-abrasive bottom surface area on which to rest, as opposed to a large tank volume (van Beek *et al*., 1990); pelagic schooling species require volumes sufficient to maintain normal schooling behaviour (e.g. Misund and Beltestad, 2000); scombrids require a high water flow [e.g. bluefin tuna (*Thunnus thynnus*) in aquaria]; and cannibalistic or aggressive species, such as nephrops, may require isolation from each other (e.g. Wileman *et al*., 1999; Castro *et al*., 2003).

Containment facilities can be broadly categorized into two forms:

i) **Tanks or ponds:** the water holding the subject population is contained by a man-made construction. The water mass is isolated, and, therefore, water quality depends on treatment or filtration, and a flow-through or recirculation supply. Tank facilities allow

the observer to maintain a high degree of control over the subject population, and they generally also allow for the subject population to be frequently monitored. However, their volume can be restrictive, and providing representative conditions is challenging.

ii) **Cages or pens:** the subject population is contained in a volume of water, generally within a larger natural water mass, using a man-made construction (typically netting). Water quality is determined by the surrounding water mass and depends on having sufficient exchange through the cage structure. Using cages in the field makes it simpler to provide representative environmental conditions. However, finding a suitable location for the cages can be challenging, and their isolation from the observer, and their large size, can make effective monitoring of the subjects difficult.

The development of appropriate containment facilities will often require preliminary investigations to assess their effectiveness. These are best undertaken in association with the development of captivity (method) controls (Section 6). A pilot study prior to the main experiment, assessing the suitability of the cages or tanks, is often valuable and may prevent costly investments in unsuitable equipment.

Examples of tanks, cages and pens are shown in figures 9.1–9.7. When designing containment facilities for a given species, key considerations include:

- non-injurious, non-toxic construction and materials (Section 9.2.3.1);
- volume/surface area (Section 9.2.3.2);
- stocking density (Section 9.2.3.3)
- stable and appropriate environmental conditions (Section 9.2.3.4);
- sufficient water quality and exchange (Section 9.2.3.5);
- water movement (Section 9.2.3.6);
- lighting conditions: intensity, spectrum, and periodicity (Section 9.2.3.7);
- shelter for the subjects (Section 9.2.3.8);
- feeding requirements of the subjects (Section 9.2.3.9);
- exclusion of predators (Section 9.2.3.10); and
- methods to facilitate monitoring with minimal disturbance (Section 9.2.3.11).

9.2.3.1 Construction and materials

The design and materials used to construct the cage or tank (and associated handling equipment) should minimize the risk of injury and physiological distress. For example, there should be no sharp edges or abrasive materials (e.g. knotless netting is preferred over knotted). Where it is anticipated that subjects may strike tank walls because of either their own activity or the movement of the vessel on which the tanks are kept, it may useful to install cushioning materials on the tank walls and/or use circular tanks. Care should be taken to ensure that the construction materials are non-toxic (see example in Table 9.1).

Where subjects come into contact with surfaces in the containment facility (e.g. flatfish), the risk of injury from contacting those surfaces should be minimized. In some cases, access to familiar substrata can be provided (e.g. sand, gravel), to minimize captive effects (e.g. Sangster *et al.*, 1996; Wileman *et al.*, 1999). Tank or cage shape is also important. For example, pelagic fish need cylindrical or circular cages for schooling; while elongated tanks may exacerbate water movement induced by vessel motion (Section 9.2.3.6).

Figure 9.1. Tank arrangement used in the UK (manufactured by Precision Pipework Ltd., Lowestoft UK). Top: tank array; middle: pump unit; bottom: individual tank. Credit: Centre for environment, fisheries and aquaculture science (Cefas), UK.

Figure 9.2. Cages used for studying *Nephrops* survival [reproduced from Méhault *et al.* (2016), and Wileman *et al.* (1999)].

Figure 9.3. Cages and holding tanks used in Dutch experiments (reproduced from van Marlen *et al.*, 2013).

Figure 9.4. Top: on-board monitoring rack (152 cm L × 59 cm W × 160 cm H) with 16 independently-mounted, flow-through, 30-l monitoring containers (60 cm L × 40 cm W × 12 cm H. [Credit: Flanders research institute for agricultural, fisheries and food research (ILVO)]. Bottom: similar racks used in the UK [Credit: Centre for environment, fisheries and aquaculture science (Cefas)].

Figure 9.5. *In situ* sea cages for monitoring discarded catches out at sea on the seabed (Credit: Wageningen marine research, The Netherlands).

Figure 9.6. Captive holding facility to monitor survival of discarded Norway lobster [Credit: Swedish university of agricultural sciences (SLU), Sweden].

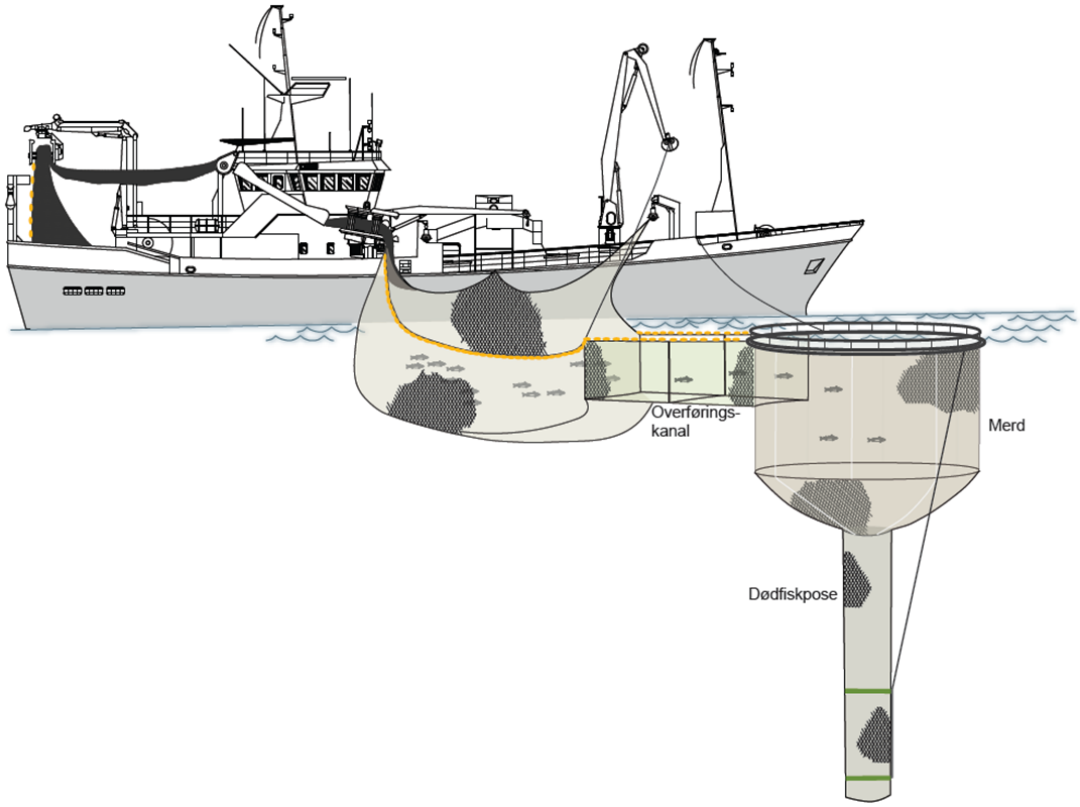

Figure 9.7. Cage setup used in Norwegian purse-seine survival studies (reproduced from Huse and Vold, 2010).

Table 9.1. Properties of the materials used in the construction of holding tanks for crustaceans (reproduced from Jacklin and Combes, 2007).

Material	Suitability	Comments
Glass	Yes	Ensure that sealants are non-toxic. Use ones made for aquaria – others are toxic.
Aluminium	Yes	Can be expensive. Needs to be suitable grade for seawater. Will it have electrolytic action with any other parts in the system?
Copper, bronze, brass, lead zinc ie. sacrificial anode	No	Heavy metal issue with seawater and foodstuffs.
Fibreglass and epoxy resins	Yes	Caution is needed with polyester resin as it may leak styrene into the holding system. If it is a recirculating system, styrene can accumulate to toxic levels. Epoxy resin leaches fewer chemicals, but is more expensive than polyester. In either case, the tank system should be thoroughly flushed to ensure removal of all toxic lactates from the resin.
Wood, bare natural and plywood	Yes	Wood is a good material for making a cheap tank for a trial. Marine plywood products are ideal. Ensure that glues are non toxic.
Wood treated with preservative	No	Could be used to provide structure, but not suited for containing water due to preservatives.
Paints	Some	Talk to paint manufacturers to ensure that the product is suited to the application, that it is non-toxic, and if possible food grade.
Plastics, often used for pipework	Some	Ensure that any plastic cements/glues are non-toxic. Food-grade vinyl tubing or PVC pipe. Ensure that glue does not collect inside the pipe joints and after drying flush with freshwater.
Cement/concrete/block work	Yes	Suitable for open through-flow systems. Cheap and readily modified, but will need to be lined for closed recirculating systems due to potential bleaching of aluminium and other metals that may be in the sand used to make the concrete.
Netting	Yes	Rigid plastic netting or fishing netting taut over a frame can be used to provide "shelving" within a tank to increase the floor space for crustaceans, while still allowing water to circulate. Check for toxicity.

9.2.3.2 Volume/surface area

An appropriate space for the experimental subjects should be provided, both with respect to the needs of the individual subjects, and to the size of the population to be accommodated (i.e. stocking density; Section 9.2.3.3). For example, schooling, pelagic fish [e.g. herring (Clupea harengus), mackerel (Scomber scombrus), and tuna] are likely to require large volumes to ensure that the containment space does not confine their natural swimming behaviour or school structure. In contrast, demersal species [e.g. plaice and sole (Solea solea)] require adequate surface areas on which to rest, which can be achieved by providing layered shelving within the holding tank (e.g. Revill et al., 2013).

9.2.3.3 Stocking density

Stocking density refers to the number of fish held within the containment facility. It is unlikely that a captive observation experiment will be able to provide natural stocking densities for the experimental subjects. In most cases, stocking density is likely to be artificially high. However, it should not be so high that it is detrimental to the vitality of the subjects. Stocking density should also not compromise water quality within the tank or cage, particularly with respect to oxygen depletion and the accumulation of waste products (see Section 9.2.3.5. Tolerance to crowding varies among species, and even within species depending on their maturity and physical status.

There are few standard recommendations for optimal stocking densities for captive observations, since these will be dependent on the species, their status, and the characteristics of the containment facilities. Information from aquaculture, sufficient investment in preliminary trials, and developing suitable methods for controls will inform researchers on this issue.

9.2.3.4 Stable and appropriate environmental conditions

It is important to ensure that environmental conditions within the cage or tanks (e.g. temperature and salinity) are representative of the habitat to which the subject should be released (i.e. its preferred habitat). Ideally, environmental conditions should also be stable in order to minimize any confounding effects on the survival estimates, unless instability is a particular feature of the subject's normal habitat. Moreover, these conditions should be replicated and monitored in each cage or tank.

Where cages are being deployed to the receiving habitat, they should be lowered and recovered gently, ensuring that there is no excessive water flow which could stress the animals. Simulating the water pressure at the depth to which the subject would return on release can be considered for tanks. Changes in key environmental parameters (e.g. depth, temperature, salinity) should be monitored throughout the period of captivity (e.g. Breen *et al.*, 2007; Knotek *et al.* 2015).

9.2.3.5 Water quality and exchange

Insufficient oxygen and elevated toxins can kill the experimental subjects. However, even at sublethal levels, the stress induced by these factors is likely to affect subsequent survival. There should be sufficient water exchange within the cage or tank to ensure that oxygen levels are not depleted, and that biowaste products (particularly ammonia) do not accumulate. Moreover, where possible there should be regular monitoring of concentrations of oxygen and key biowaste products.

Water exchange in tanks should be designed in such a manner that intertank contamination is avoided. Ideally, each tank should receive its own independent water supply. Cages may require cleaning to ensure that any growth on netting material does not compromise water exchange.

9.2.3.6 Water movement

Water movement within the containment facility can be induced naturally by tidal water currents through the cage, or artificially via a water-exchange system. While some water movement is necessary to ensure water quality (Section 9.2.3.5) and promote natural swimming behaviour, excessive movement can induce additional stress. It is recommended that water flow within the containment facility is continuously recorded, particularly in cages.

Stress can be induced by the movement of the vessel on which tanks are installed. This can be partly relieved by tank design, such as sealed tanks with no air spaces, small round tanks and/or

the use of baffles to restrict water movement. The tanks should also be securely fastened in a position on the vessel where the ship's motion is minimal.

When using cages, sites should be selected that are sheltered from significant tidal currents and the prevailing weather. Floating cages drifting with water currents, as opposed to being anchored, can be considered (Huse and Vold, 2010). Cages should be deployed and recovered gently, ensuring that any induced water flow does not stress the subjects.

9.2.3.7 Lighting conditions: intensity, spectrum, and periodicity

Many aquatic species are adapted to light intensities much lower than those experienced at the surface (Johnsen, 2012). Moreover, the subject's natural light will have a periodicity and spectrum that will be specific to its natural habitat (Johnsen, 2012). To minimize captivity stress, holding conditions should attempt to simulate natural light levels and patterns. If held in tanks, artificial lighting can be used, with appropriately coloured or opaque construction materials, to replicate natural lighting conditions.

9.2.3.8 Shelter for the subjects

Some species naturally seek and require shelter, and will likely become stressed without them (e.g. *Nephrops*). Provision of a suitable artificial shelter can alleviate this problem (e.g. Wileman *et al*., 1999).

9.2.3.9 Feeding requirements of the subjects

Most adult aquatic species can survive several weeks without food, especially at lower temperatures. However, when observing the experimental subjects for a prolonged period, it may be necessary to provide food to meet the subject's nutritional requirements. A review of the life history of each species will provide the needed information to determine feeding requirements. Providing food may also alleviate predation and cannibalism within the captive population. The feeding status of a fish can be a useful measure of its vitality and stress status (e.g. Breen, 2004). Finally, feeding will increase the subject's oxygen requirements and the production of biowaste products; and, therefore, water quality will need to be maintained accordingly.

9.2.3.10 Exclusion of predators

Where there are likely to be intra- or interspecific interactions (e.g. cannibalism, competition, and predation), it may be necessary to exclude some species or larger individuals, or to have segregated facilities. Cages deployed in the subject's natural habitat can attract predators and scavengers that can stress the subjects, enter the cage and attack live subjects, or scavenge on dead specimens (e.g. seabirds, starfish, crabs, and sea lice). Efforts should be made to exclude these animals (e.g. floating cages should be covered by netting or a lid to avoid predation by seabirds.). In addition, regular monitoring and removal of dead animals (e.g. using divers) can be used to limit the attraction of scavengers.

9.2.3.11 Facilitate monitoring with minimal disturbance

Monitoring of the subjects should be conducted in a way that minimizes stress on them. Remote monitoring technologies (e.g. video cameras) can be used to monitor mortality and vitality without adding to captivity stress by disturbing them (e.g. Ingolfsson *et al.*, 2007). Closed-tank facilities allow assessments of the physiological status of the subjects (e.g. measuring excreted levels of cortisol).

9.2.4 Monitoring

This section describes the practical aspects of monitoring the fish during the captive observation period, including defining when a subject is dead and the frequency of monitoring events.

9.2.4.1 Characterizing subjects as dead

Characterizing a subject as dead can be subjective, so a consistent protocol is necessary (e.g. Benoît *et al.*, 2013; sections 6 and 8). Clearly defined, measurable, and validated characteristics of a "dead" subject should be established prior to the commencement of the survival experiments (e.g. lack of respiratory or gill response, swimming activity, onset of rigor mortis, lack of reflexes or response to stimuli, or colour of gills).

9.2.4.2 Removal of dead specimens

Subjects that are characterized as dead should be removed as quickly as possible to reduce the risk of disease and/or the attraction of predators and scavengers; and their time of removal should be noted. In tanks, dead subjects can typically be removed with properly designed landing nets (i.e. those used by aquarists or anglers). Additional stress to the remaining living subjects should be minimized. When storing fish in cages, various solutions designed for removing dead fish in aquaculture are available (e.g. Sangster, 1991; Piggott, 2013). For underwater cages, divers or ROVs may be deployed.

9.2.4.3 Observations

Observation of the captive subjects should be a compromise between obtaining accurate data on the occurrence of death, with timely removal of dead specimens, and the disturbance and stress caused by the observation. Monitoring should be done with minimal disturbance (see Section 9.2.3.11.

Regular monitoring is required to generate a cumulative mortality profile. Monitoring should ideally continue until mortalities cease and the cumulative mortality profile reaches a plateau or symptote (see following text). Mortality rates may stabilize only to increase after a lag period. Mortalities closer to the time of discarding are likely due to the capture-and-discarding process, whereas mortalities towards the end of monitoring might be due to containment. Controls should be used to establish any method-induced mortality (Section 6).

Monitoring should ideally be carried out for as long as it takes to explicitly observe the treatment-induced mortality. A typical cumulative mortality curve has an asymptotic shape (Benoît *et al.*, 2013). The experiments should therefore continue until the mortality approaches the asymptote (see also Asymptotic Survival, Information Box 12.1). This may take days or weeks, depending on the species and treatment. In practice, however, the duration of the monitoring often has to be a trade-off between scientific needs, available resources (sea time, budgets, available tank time), and occurrence of confounding mortality not associated with the initial treatments.

A bimodal mortality may occur in some cases, such as in herring (A. Vold and R. E. Olsen, pers. comm.). In such cases, untreated controls are needed to determine whether a second peak of mortality is caused by the initial treatment, or is attributable to a captivity effect. Furthermore, there may be cases when the mortality rate does not stabilize. In such cases, it is difficult to deduce if the mortality is related to the treatment or to captivity.

In previous captive survival assessments, monitoring has typically been done every 24 h. This provided a balance between the level of disturbance, resource requirements, and data generation. This guidance recommends that more frequent monitoring is conducted in the first 24 or 48 h after discarding, since this is the period during which the highest mortality rates are

often seen. In some experiments, only endpoint mortality can be monitored, since daily sampling of mortalities is logistically difficult or even impossible, (e.g. Ingólfsson *et al.*, 2007; Huse and Vold, 2010).

9.2.4.4 Variables to be measured

The variables to be measured will depend on the study and the ability to sample specimens during the assessment. Length, weight, and sexual maturation of the subjects are important variables to understand the susceptibility to mortality of the population. External injuries may also be recorded, keeping in mind that handling of the fish and post-mortem processes may cause damage. Details on methods to visually assess the vitality of fish are given in Section 8. Laboratory studies can utilize a much wider range of analytical techniques [e.g. analysis of stress axis components (cortisol, plasma ions, catecholamines, and glucose) and hypoxia indicators (lactate)]. In tank studies, non-invasive techniques like measuring levels of cortisol and ammonia in the water are well suited. Measurements of water quality variables like oxygen content, ammonia, salinity, temperature, and current velocity are useful for establishing how well the containment reflects the natural environment, and to correlate changes in conditions with mortality rates.

9.3 Summary

In captive observation, experimental subjects are retained in captivity for a period of time to monitor their survival. Captive observation can be conducted in field assessments or in the laboratory. Laboratory-based assessments can be used to investigate the effects of isolated variables on the behaviour, physiology, and survival of subjects under controlled conditions. Stressors representative of normal fishing conditions are usually not reproducible in the laboratory and, therefore, these assessments are considered less suitable for generating fishery-specific discard survival estimates. In field assessments, experimental subjects are collected under representative fishing conditions and then transferred to containment facilities for captive observation.

Captive-observation techniques may introduce biases to survival estimates. They do not include predation effects on survival of discards, nor some stressors that would otherwise have been experienced by the fish had they been discarded back into the sea, and so may overestimate the true survival rate. In addition, they may add handling and captivity stressors and, therefore, can underestimate the true survival rate.

The design of an effective captive-observation assessment depends on six key elements:

1. Obtaining a subject population that is representative of normal fishing conditions.
2. Transfer into captivity under conditions ideally representative of those the discarded fish would normally experience during release.
3. Containment in appropriate conditions in tanks, ponds, or cages, which ideally suit the species-specific biological and behavioural needs. Key characteristics to be considered include:
    - Construction and materials
    - Volume/surface area/stocking density
    - Stable and appropriate environmental conditions
    - Sufficient water quality and exchange
    - Water movement

- Shelter
- Nutrition
- Exclusion of predators
- Facilitate monitoring with minimal disturbance
- Dead subjects should be removed as quickly as possible.

4. Monitoring for ideally as long as it takes to explicitly observe the treatment-induced mortality, and at sufficiently regularity to generate a cumulative mortality profile for estimating asymptotic survival (S^A).
5. Clearly defined, measurable, and validated characteristics of a "dead" subject.
6. Controls should be used to establish any method-induced mortality.