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Bruno Leroy, Joe Scutt Phillips, Joanne Potts, Richard Brill, Karen Evans, et al.. Recommendations towards the establishment of best practice standards for handling and intracoelomic implantation of data-storage and telemetry tags in tropical tunas. Animal Biotelemetry, 2023, 11 (1), pp.4. 10.1186/s40317-023-00316-3 . hal-04032791

Leroy *et al. Animal Biotelemetry* (2023) 11:4
https://doi.org/10.1186/s40317-023-00316-3

Animal Biotelemetry

COMMENTARY

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# Recommendations towards the establishment of best practice standards for handling and intracoelomic implantation of data-storage and telemetry tags in tropical tunas

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**Abstract**

Archival (data-storage) and telemetry (acoustic and radio) tags are commonly used to provide data on the behavior and physiology of organisms, as well as data on their surrounding environment. For fishes, it is often advantageous to implant tags in the peritoneal cavity (i.e., intracoelomic implantation). The literature on best practices is limited for marine species, and near absent for tunas despite their regular application. We identify recommended practices using laparotomy in tropical tuna species following observations from thousands of tags implantations undertaken during implementation of several tagging programs across the Pacific. These recommended practices include descriptions of preferred tagging stations and equipment, fish selection, surgical procedures, and return of the fish to the wild. While these recommended practices were developed specifically for tropical tuna species, they are also likely applicable for other pelagic fishes. We present these guidelines to guide and promote the development of best practices for such procedures on pelagic species.

**Keywords** Electronic tag, Body-cavity tag, Laparotomy, Tagging protocols, Tuna

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## Background

Data-storage and telemetry tags (emitting either radio or acoustic signals) are designed for intracoelomic implantation (hereafter collectively referred to as "body cavity tag" or BCT) and are battery-powered electronic devices encased in biologically inert resin, stainless-steel, or ceramic. Acoustic BCTs have been deployed in marine fisheries research since the 1960s [6, 10, 16, 28, 103] and are now commonly used to monitor movements and behaviors at locations of aggregation or confinement [33, 34, 37, 43, 52, 81, 95] or along corridors of seasonal movement [14]. Data-storage BCTs (referred to as Archival or Data Storage Tags) were developed in the 1990s [7, 39]. Their function is to record a fish's internal temperature, environmental conditions (e.g., depth, temperature, and light level) and, like acoustic BCT, are commonly used in studies designed to (1) quantify pelagic fish behaviors and movements; (2) measure their associations with environmental factors or fish aggregating devices; and (3) inform stock assessments and management interventions [1, 82, 87].

Methods for intracoelomic BCT implantation have largely evolved from studies of freshwater fisheries [23]. Standard protocols [23, 41, 42, 48, 62, 63, 91, 97, 100] include: (1) selection of suitable individuals for implantation; (2) placement of selected fish in an anesthetic bath until the fish is deemed to have received a predetermined level of analgesia, paralysis or unconsciousness; (3) transferring the anesthetized fish to a V-shaped surgical cradle for BCT implantation (typically through a mid-ventral incision in a location posterior to the pelvic girdle); (4) maintaining gill irrigation throughout the surgery; (5) suturing the incision closed once the tag is positioned; (6) transferring the fish to a bath to facilitate recovery from anesthesia; and (7) when deemed fully recovered release back into the wild. Although many of these protocol components remain valid when tagging tunas, many need to be adapted to the specifics of these species and the tagging environment. Here, we describe practices we recommend for BCT implantation in wild-caught oceanic tunas.

BCT implantation in tunas is complicated because of tunas' remote oceanic habitats and physiology. Although some tunas (Family: Scombridae, Subfamily: Scombrinae, Tribe: Thunnini) are accessible near shore, their core habitats are oceanic regions distant from land. This necessitates the use of vessels that can operate in open ocean environments, are able to travel long distances, and have the capacity to capture tunas. This often restricts vessel choice to commercial fishing vessels, which generally have limited options for surgery table placement and designs. The unique physiology of tuna species also makes it difficult to maintain live individuals out of the water without life-threating consequences, especially in tropical and equatorial environments. Tunas are obligate ram ventilators, and rely on their forward motion to force water through their mouth and over the gills [11]. In addition, tunas are generally slightly negatively buoyant and as a result need to maintain minimum sustained swimming speeds required for achieving hydrostatic equilibrium [56, 57]. Because of their limited tolerance of interruptions to water flow over their gills, individuals for BCT implantation can be unventilated for only between 30 s and 3 min before stress and oxygen depletion compromise their post-release survival [79]. Tunas are also thermo-conserving (i.e., they maintain swimming muscle temperatures above ambient) and regulate their body temperatures through physiological mechanisms and by rapid vertical movements through the thermocline [30, 31, 44]. Unlike other teleost fishes, tunas therefore risk overheating if they are not able to regulate their internal temperature through such movements [9, 44].

The capture and holding of tunas are also challenging [85]. Holding individuals in tanks while at sea before and after BCT implantation is generally impractical. Experience in the transport of tuna on vessels has shown that tunas cannot turn at tight angles, so tunas require large (minimum 2 m diameter) circular or oval tanks to facilitate the continuous swimming required for ventilation and hydrostatic equilibrium [66] and ensure that any collisions with tank walls are minimized. Such large tanks are difficult to install on commercial fishing vessels typically used for tagging. Additional stresses due to confinement in tanks may also have a negative impact on post-release survival [40]. Tuna also school as part of their behavioral response to avoid predation [36, 74], so extended time in surgery is likely to isolate an individual from its school. As such, holding tunas either before or after surgery is unlikely a viable alternative to their quick capture, handling and release. The implantation of BCTs in tunas must, therefore, be executed quickly to minimize the risk of fatal physiological changes (i.e., oxygen deficiency and internal temperature rise) and isolation from the school upon release.

## Recommendations towards establishment of best practices

Most projects involving BCT implantation in wild caught and released fish assume that tagged individuals have similar fates and behaviors relative to untagged conspecifics, thereby allowing species-specific ecology and movements to be inferred from the behavior patterns of tagged fishes [70, 89]. The surgical procedure used for intracoelomic BCT implantation using laparotomy has the potential to bias subsequent inference if it permanently alters the survival, behavior, or overall health of the animal [12]. The effective implantation of a BCT, and subsequent survival of tagged fish, is dependent on appropriate planning and implementation from capture to release. Thorough planning ensures circumstances that could result in an adverse impact on the wellbeing of an individual are identified their likelihoods and consequences estimated and mitigating measures put in place to ensure they are avoided. The practices described herein are designed for field application on tunas (or similar pelagic species) caught and released from commercial fishing vessels, based on our collective experience over the past decades. They are based on the premise that approaches that minimize stress will result in a faster return to normal physiological conditions and homeostasis, and thus minimize both rates of post-release mortality and compromised growth, development, disease resistance, behavior, and/or reproduction [86].

Our choices of recommended practices are for those that maximize animal wellbeing and minimize (or avoid) harm, pain, and distress caused by handling and the surgical interventions necessary for intracoelomic BCT implantation. They ensure that harm, pain or distress are not greater than those likely to be experienced by individuals in the wild [8, 26, 27, 67]. We also deem such practices to necessarily include those that ensure all procedures (from capture to release) are performed competently and that minimizing harm, pain and distress take precedence over completing the procedure itself.

#### Fish capture and handling

In addition to the surgical methods involved in BCT implantation, the probability of post-release mortality is influenced by handling procedures throughout all components of the tagging process (e.g., [4, 13, 54]). While handling-induced stress is species specific [75], the method and consequences of capture have been identified as important influences on the subsequent survival of tagged tunas [45]. The poor condition of tuna caught using purse seine methods typically eliminates the use of these vessels for capturing individuals suitable for BCT studies [55]. Fishing methods more conducive to facilitating the capture of fish in good condition include those associated with hook and line, pole and line and longline gears. However, careful choice of fish is still required. Individuals caught on pole and line fishing gear with eye damage, impact trauma, excessive bleeding, or mouth or esophageal damage due to hooking have lower rates of survival than conspecifics that did not suffer one or more of these traumas [45]. Similarly, fish caught and tagged on commercial longline vessels have reduced survival the longer the time-period is between hooking and retrieval [61, 71, 73]. Disruption of physiological homeostasis associated with capture is also common in fishes (e.g., [35, 60, 94]). For example, elevated plasma cortisol, lactate, and plasma ion levels have been recorded as are common when times from hooking to sampling (i.e., “fight times”) exceeded 10 min (e.g., [53, 58, 90]). Whether these elevated physiological conditions translate into increased rates of post-release mortality is less clear (e.g., [29, 60, 94]). Estimates of post-release mortality suggest, however, that removal of fish from the water can decrease the probability of survival (e.g., [21, 83]).

*Recommended practices* in fish handling include identifying factors that contribute to the level and duration of harm, pain, and distress, and implementing steps to avoid or minimize these [2]. Applying these practices to BCT implantation in tunas includes diligent approaches to fish selection immediately upon capture. Fish should be rejected if there is:

1. Observation or other evidence of a broken fin or jaw,
2. Muscle trauma associated with an impact on the boat hull, gunnel or deck during capture and handling,
3. Protuberant eye (barotrauma) or blood presence in the eyeball indicating hook damage,
4. Gill damage indicated by bleeding from the gill plates,
5. Any other excessive bleeding (constant flow),
6. Significant hook damage to palate or tongue,
7. Rapid loss of color,
8. Constant mouth gape (often associated with gill flaring and/or constant tail flapping),
9. Evidence of exhaustion due to prolonged fight time (lack of vigorous swimming), and
10. A taught (or swollen) appearance of the skin and muscle surrounding the peritoneal cavity indicating full stomach due to ingestion of excessive amounts of seawater or an overinflated swim bladder due to barotrauma.

Individuals showing signs of minor stress or injury can be returned to the water and released as soon as possible, whereas individuals showing any signs of the above listed stress or injury should be euthanized immediately.

*Recommended practices* also include ensuring that the gear and methods for landing a fish minimize harm, pain or distress. Pole and line is the predominant method used to catch tunas for BCT implantation. Because the size and shape of the hook(s) used influence the location and severity of tissue damage, hooks should be barbless (or the barbs crushed) to facilitate hook removal. Likewise, the gear should be adapted to minimize the fight time and tissue damage. Thus, the size of the targeted fish should be considered in hook sizing. The fish should also be caught near the surface to avoid barotrauma. For tropical tuna species, pole and line gear allow fish of up to a dozen kilograms to be safely lifted out of the water and directly placed on the tagging cradle. Above that size, a scoop net with knotless webbing (Fig. 1) or a sling device designed for the purpose (Fig. 2) should be used to lift the animal out of the water to avoid further injury and minimize stress during landing. Both gears work efficiently for large tuna but the choice of best gear for a particular fishery will depend on vessel, deck gear and crew

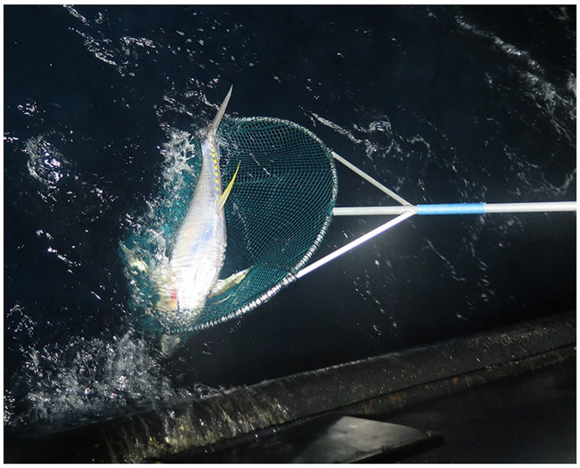

**Fig. 1** A 20 kg yellowfin tuna being lifted onboard using a handheld aluminum framed scoop net with knotless webbing

**Fig. 2** A sling designed to lift and release large tunas

experience. Fish lifted directly by line or with a scoop net should be placed on a non-abrasive, soft, and wet surface (like a vinyl covered 3-cm-thick foam mattress) covering the deck to avoid further skin abrasion. The fish is then transferred by hand from the mat to the cradle (see *surgery platform*). For fish lifted with a sling, surgery is usually implemented in the sling to avoid hazardous manipulation of large animals. Tagged fish are then safely released within the sling. We also note that tuna should not be carried or picked up by its caudal peduncle. Tuna spinal columns have evolved to function under compression not tension, and the associated connective tissue rapidly fails under the latter. When being moved, tunas should be cradled either by remaining flat on their sides (larger individuals) or by a hand under their pelvic fins. The weight of an individual should be supported across the entire body to avoid stress and injury. To minimize stress, the tuna's eyes should be covered as soon as possible by a seawater-soaked chamois (described in *Surgery Kits*) following the fish being placed in the tagging cradle with the chamois remaining in place until release. Tuna may need to be lightly restrained for the minimum time needed to complete BCT implantation by trained and practiced crew to prevent injury to either the tuna or the surgery personnel. If fish restraint requires more than a slight effort, and result in an excessive amount of stress, the operation should be aborted.

## Anesthesia and analgesics

*Recommended practices* for freshwater species and many marine species during surgery usually require the use of local and general anesthetics, analgesics and/or sedatives [60, 96]. Criteria for choosing an appropriate agent(s) include:

1. Suitability for the species, age, developmental stage, and physiological status;
2. Appropriateness for the type of procedure;
3. Administration causes minimal distress; and
4. Capacity to monitor effectively the fish's wellbeing throughout the operative and post-operative periods until full recovery [100].

Use of general anesthesia should also consider the feasibility to monitor that: (1) an adequate level of anesthesia is maintained; (2) physiological disturbances are being minimized; and (3) potential complications (e.g., cardiovascular and respiratory depression) can be detected and managed both during surgery and post-operatively [68].

While the return of equilibrium (i.e., the ability to maintain an upright swimming posture) is the usual indicator for recovery, the physiological and behavioral effects of the anesthesia can extend well beyond this point [17, 38, 47, 76, 78, 104]. Pelagic predators such as billfishes, sharks and toothed whales often follow and feed on tuna schools, and may aggregate near fishing vessels during operations. As such, it is important that there are no residual effects of the anesthesia that would increase rates of predation.

We contend, however, that the use of anesthetics and analgesics are not within *recommended practices* for

BCT implantation in tunas for multiple reasons. First, some tropical tuna species (e.g., skipjack tuna; *Katsuwonus pelamis*) are known to have low survival rates after being anaesthetized [15]. Second, having holding tanks of sufficient size to allow individuals to fully emerge from anesthesia is highly impractical on the majority of vessels used for tuna tagging (see Background).

Finally, there are legal restrictions and obvious ethical and safety concerns surrounding the use of anesthetics and analgesics in fish that may be captured for human consumption [e.g., 84, 100].

We instead contend that dorsoventral inversion to induce tonic immobilization conditions [51] is more appropriate. It allows for rapid handling, BCT implantation, and release back into the water. We recognize, however, that tonic immobilization does not induce loss of sensation [91], but argue that its benefits associated with the reduced stress associated with rapid return to the water supersede the impracticalities of anesthesia or sedation. Our field observations indicate, moreover, that the incisions required for BCT implantation are equivalent to, or less than, non-lethal predation damage observed in larger tunas and billfishes [69, 72].

If either anesthetics, analgesics, or sedatives are used, records should be kept on the products used, methods used for administering, any complications and the individual's recovery so that the efficacy can be evaluated [18].

### Surgery practices

Effective BCT implantation is reliant on a well-placed surgery platform to ensure that: (1) operations are quick, safe and efficient; (2) that aseptic practices are adhered to (to the extent practicable); and (3) the procedure is terminated at the first signs of deterioration in fish condition to minimize pain and distress. The latter implies that planning for surgery includes defining intervention points (to release the fish back to the water) and endpoints where the animal must be humanely euthanized.

#### *Surgery platform*

Adapting the surgery platform to the vessel and the species to be tagged (i.e., "fit for the purpose") significantly reduces the time taken for the BCT implantation. Our collective experience is that a V-shaped (45° angled) cradle facilitates tonic immobilization and safely maintains fish in a ventral side-up position (Figs. 3, 4). The cradle should be padded with closed-cell foam, and the padding covered with smooth vinyl surfaces to reduce skin abrasion. A scale with 1-cm-length increments printed on the cradle pads facilitates the quick measurement of fish length, a commonly recorded metric that ensures

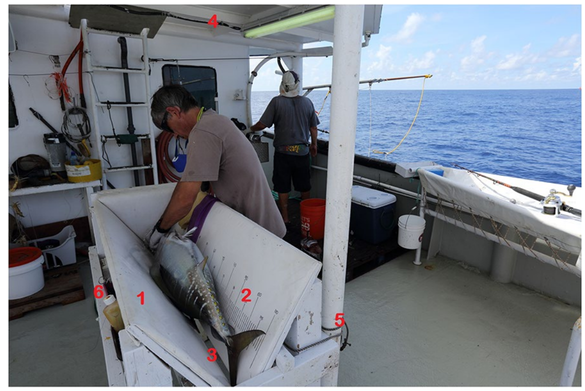

**Fig. 3** Cradle with (1) fitted and padded smooth vinyl surfaces to safely restrain the fish and avoid skin abrasions, (2) a ruler printed on the pads for rapid fish length measurement and to reduce the time the fish is out of the water; (3) a gap at the base to allow possible passage of dorsal fins and tail; (4) positioning of the cradle to provide protection from sea-spray, inclement weather, and direct sunlight; (5) stable legs that ensures the cradle is at a comfortable height for BCT implementation; (6) easy to clean (and detachable) surgical and tag trays to store all surgery tools and BCTs fixed in ergonomically and easily accessible locations

**Fig. 4** BCT implantation into a yellowfin tuna demonstrating (1) an insertion point for a sea-water hose to irrigate fish gills; (2) the positioning the fish ventral side up with care not to damage any fins; (3) positioning the tail to avoid possible contact with hard parts of the cradle frame when the fish's length is larger than the cradle length; (4) securing of the cradle on the vessel working deck; and (5) the cradle design with stable legs that ensures it is at a comfortable height for BCT implementation

that fish are of adequate sizes for the size of the tag are considered and also allows for the calculation of growth rates when coupled with measurement on recapture. The cradle design should include a gap at the base (i.e., at the vertex of the V-shaped cradle; Fig. 3) to preclude damage to the dorsal fins and tail, and an insertion point drilled through the end plate for a sea-water hose to irrigate fish gills (Fig. 4). During BCT implantation the fish should be placed ventral side-up on the wetted berth of the cradle (taking care not to damage the pectoral fins), with the head placed next to the sea water hose (Fig. 4). For fish larger than the surgery cradle, care should be taken with the tail to avoid possible contact with hard parts of the frame. In this situation, any measurement of fish length should be done using calipers either immediately before or after BCT implantation.

The frame should be secured on the deck, with stable legs that bring the cradle to a height that is ergonomically comfortable for the operator undertaking the procedure. Clean trays to store all surgery tools and BCTs should be fixed in ergonomically and easily accessible locations. Positioning of the cradle to minimize handling of fish between landing and transfer to the cradle, and from the cradle to release, also reduces the risk of injury to the fish during transfers and supports efficiencies in the time taken to undertake procedures. The cradle should be positioned, to the extent practicable, such that it is protected from sea-spray, inclement weather, and direct sunlight. Exposure to the former increases stress and often reduces the probability that the fishes will remain still during BCT implantation. Excessive fresh or salt-water sprays directed into the surgery site should be avoided as they can complicate BCT implantation and reduce the efficacy of disinfectants.

### *Surgery kits*

Choice of the equipment used for safe BCT implantation is crucial to optimize overall operational success. In our experience, familiarity and comfort with the equipment is critical to achieve quick and uncomplicated BCT implantation. We recommend that a minimum of equipment is used at any one time to avoid cluttering of the tagging cradle and its immediate surrounds. Surgery cradles may necessarily be placed near the working deck of the vessel (to provide immediate access to landed fish) which can become crowded during fishing activities. Vessels can also be operating in moderate to rough seas which increases the instability of any storage of equipment on the surgery cradle. Minimizing the presence of equipment therefore reduces the risk of loss or damage (to both the equipment and fish) (Figs. 5, 6).

To minimize fish stress and maximize immobilization, the surgery kit should include a cover for the fishes' eyes. We have found a wet soft chamois (leather or synthetic) sheet is most suitable. This material is thick enough to provide a good light barrier, has sufficient frictional properties to prevent it from easily slipping off the fish's head, but is smooth enough that it does not damage the cornea. It is also easily cleaned between surgeries in (preferably) fresh water, as any blood is quickly washed out.

**Fig. 5** Surgery tools: scalpel, knives, sharpening stone, needle holder (forceps with cutting capacity) and suture material

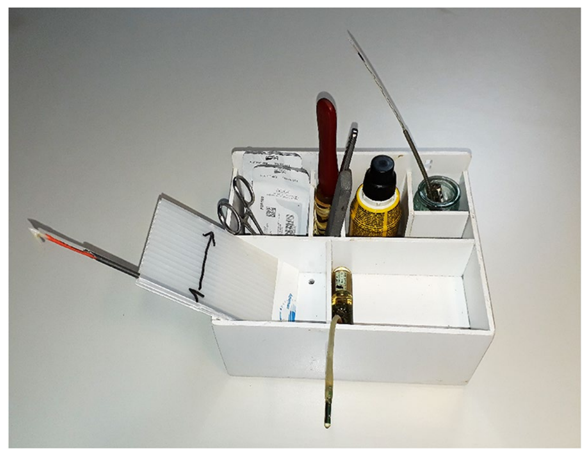

**Fig. 6** Customized plastic compartmented container holding BCTs, plastic identification tags and surgery tools

Either a scalpel blade and handle or a very sharp paring knife can be used for making in initial incision in the body wall over the peritoneum. Generally, a sharp paring knife is a safer tool on a moving or rocking vessel, but requires frequent sharpening. Forceps with cutting capacities, such as Olsen–Hegar needle holders with suture scissors are a crucial tool that must be part of the equipment and is used to handle the suture material (see E.) used to close the incision.

### *Aseptic practices*

Pathogens may be transferred to fish from fish handling equipment, surgical instruments, suture material, the BCT [63, 65, 100], or directly introduced through the surgical incision [20, 63] with negative outcomes for tagged fish. Aseptic practices increase the probability of full and fast recovery from surgery and include: (1) the use of single-use surgical gloves and clothing [63]; (2) the use of a fresh set of sterile surgical instruments for each surgery [24, 98, 100]; (3) avoiding water entry into the coelom [100]; and (4) washing down of fish holding equipment such as cradles between surgeries [100]. While poor aseptic practices have not been directly linked to altered growth, behavior, swimming performance, feeding, predator avoidance and healing in the longer term [49, 50], minimizing the chance of post-surgery infection maximizes the likelihood of fast recovery and surgical site healing [64].

Access to electrical power is typically available on those vessels used for tuna tagging. This allows for sterilization of surgical equipment using steam autoclaves [100] or ultra-violet radiation sterilizers [99]. These are preferred to chemical sterilants and disinfectants as sterilants and disinfectants may cause adverse reactions when they contact fish tissue and are generally considered unsafe [100]. Despite this, as surgeries are undertaken in the open-air, placing surgical equipment and BCTs in a bath of povidone-iodine (or equivalent e.g., chlorhexidine) will help reduce potential airborne contamination. Both must, however, be rinsed in a sterile saline bath prior to use. To help maintain sterility of BCTs, once programmed and switched on, individual units can be stored in a sterile plastic bag until implantation. Surgery tools also need to be protected from direct sun exposure and any sea water spray, both when stored and during surgeries. The BCT must also be stored away from the direct sunlight and contamination sources, and at a temperature similar to the surrounding sea water.

As the availability of suitable individuals for tagging is reliant on fishing, fish often become available with little notice. This implies that the tagging station, along with the tagger, must be prepared to perform BCT implantation at short notice, including the need to perform multiple surgeries in succession over a short period of time. Having multiple surgery kits available, so that used instruments can be sent for sterilization between surgeries without delaying the next surgery, helps ensure that opportunities for BCT implantation are not missed.

## Forced ventilation

Once removed from the water, a fish is experiencing "apneic asphyxia". High sea surface temperature, air temperature and bright lights are also factors that can exacerbate the impact of this asphyxia. Our observations suggest that species specific tolerances can be different. Bigeye tuna, for example rarely show signs of distress for up to 2 min without gill irrigation, whereas skipjack tuna can show signs of stress in less than 30 s without forced ventilation. Yellowfin tuna also show less signs of stress if their gills are irrigated immediately. If it is deemed appropriate to irrigate the fish's gills during surgery, the diameter of the hose used should be kept relatively small (e.g., a 15-mm garden hose). Unless the hose is of a reinforced type, it is beneficial to insert a small length of rigid plastic tubing inside the hose end to keep it fully open under fish jaw pressure. The sea water output needs to be regulated to about 5 l/min (from collective experience). The hose should be inserted in the fish mouth up to a point immediately posterior of the "V" formed by the posterior ends of the upper and lower mandibles. The hose should not be inserted to a point where it is near the esophagus, otherwise there is a significant risk of the stomach filling with water. We recommend using a purposely built pump and tubing system with only plastic and stainless-steel parts to avoid any contamination to water from copper, brass, or aluminum parts that may be present within in situ vessel plumbing systems.

## BCT implantation and incision suturing

### *Ratio of BCT size and weight to fish size and weight*

The overall goal of deployment of BCTs is to monitor unbiased behaviors. Given this, the weight and design of the BCT should be such that it causes minimal interference with the natural behavior of the species and not cause an over-burden on the tagged fish. A published "rule of thumb" is that the mass of the BCT should not exceed 2% of the fish's body mass [19, 101, 102].

### *Surgery and tag insertion*

To avoid excessive harm to the fish, the shortest possible incision should be made that permits insertion of the BCT without excessive force. Incisions about 2.5 to 3 cm long are required for large archival tags, while smaller tags require incisions of 1.5–2 cm. We have found positioning of the cut in the abdominal wall about 1/3 of the distance between the anus and the base of the pelvic fins, and about 2 cm to one side of the centerline of the fish, to be an optimal location. This location is a compromise between the shape of the body cavity, the position of fragile organs (heart, leaver, swim bladder) and the cavity wall configuration; on the centerline location the abdominal lining is more fibrous and seems to be more sensitive to the cut. To avoid potential organ damage from the incision, the preferred method is to cut partially through the muscle rather than into the peritoneal cavity (where there is potential for damage to organs). Final entry is forced through the peritoneal lining by the insertion of the BCT. The trocar should be sterilized, and gloves changed between surgeries. Great care must be taken when forcing the peritoneal lining, and we recommend using a gloved finger as this minimizes the risk of cutting or rupturing a blood vessel. The final stage of BCT implantation is the critical phase and must be undertaken

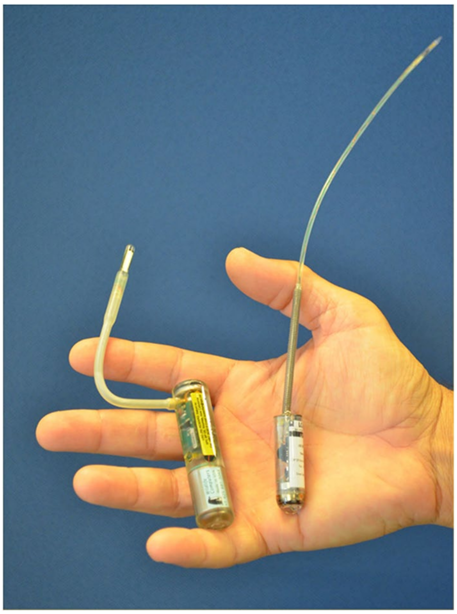

**Fig. 7** Large (left) and small size (right) archival BCTs

**Fig. 8** Incision closed by 3 staples on a skipjack tuna inserted with an archival BCT

with care so as not to harm internal organs. Presence of a full stomach (especially for smaller sized tunas) or an inflated swim bladder is likely to compromise a safe BCT implantation and should be considered as a reason to terminate the procedure and euthanize the fish. Wiping the incision point clean of any slime coat before commencing the cut is not recommended as this can increase the chances of post-release infection [93].

### Closure of incision

*Sutures* The choice of suture can influence the speed of surgery [22]. We have found that needles with reverse cutting tips can easily penetrate the skin of tunas and that curved (1/2 circle or 3/8) shaped needles, with dimensions adjusted to fish size, are needed to penetrate fully the thickness of the muscle overlying the peritoneal cavity. A 36-mm needle is suitable for fish less than 20 kg, whereas 48 mm is more appropriate for larger individuals. Synthetic absorbable monofilament sutures (polydioxanone) have been identified as potentially causing less inflammation than braided material [95], although the literature suggests little overall difference in performance between absorbable and non-absorbable suture material [22, 94]. A surgeon's knot with additional throws (3 plus 2, from informal veterinary recommendation) will ensure the ligatures remain in place on highly mobile tropical tunas. Depending on the incision size, 1 (small size archival tags or acoustic telemetry tags) or 2 sutures (larger size archival tags) are typically required to maintain incision closure (Fig. 7).

*Surgical staples* Stainless steel surgical staples (Fig. 8), placed using the accompanying staple gun, allow for rapid closure of the incision Based on a 2-3 cm incision, two or three staples are typically needed. Surgical staples may not, however, ensure that surgical wound is closed fully. In addition, it can be difficult to keep the incision closed with two fingers on one hand, while operating the staple gun with the other with the result that it is difficult to ensure that the staples properly span the incision. For these reasons, we recommend the use of surgical staples only when it is crucial to keep surgery time under one minute (e.g., skipjack tuna).

### Fish monitoring condition during surgery

In addition to the careful selection of fish for surgery, monitoring the fish throughout BCT implantation is important. *Best practices* should therefore include development of criteria to assess fish condition during BCT implantation. These assessments are best undertaken by experienced persons who can recognize specific signs of distress displayed by the tagged species. They should be supported by appropriate methods for recording observations, treatments, and actions so that the criteria and interventions can evolve through a process of continuous learning. Key indicators of excessive stress or trauma in tunas include: (1) the fish suddenly losing its color; (2) continuous mouth gaping and or flaring of the gill plates; (3) excessive tail flapping; (4) excessive bleeding from the incision; and (5) pressure being exerted by internal organs (e.g., stomach or swim bladder) causing

**Fig. 9** Bigeye tuna ready to be released after archival tag insertion. An orange plastic dart tag has been inserted behind the second dorsal fin

the incision to remain open. In these cases, BCT implantation should be terminated, and the fish immediately euthanized.

### *Addition of a conventional tag*

We have found that returns of BCTs from recaptured fish are facilitated by the presence of a brightly colored external plastic tag (Fig. 9) inserted between the second dorsal fin pterygiophores, using a standard, stainless steel tag applicator. The use of consistent color schemes for external dart tags, with relevant contact and reward information dependant on the BCT type, facilitates easy recognition during recapture and any tag recovery networks already in place. However, care should be taken not to exert pressure on the ventral surface post-surgery, which necessitates the need to insert this tag upwards into the pterygiophores, rather than turning the fish dorsal side up.

### Release back into the wild

*Recommended practices* for the release of individuals include (1) release close to the site of capture or near the school from which it was captured, (2) protection from predation immediately after release, and (3) minimize fish handling.

To maximize the likelihood of a fish returning to its school, individuals should be released as soon as possible after surgery (assuming they have not been anesthetized or sedated). Releasing the fish headfirst and keeping its eyes covered until the last moment reduces stress and from observations appears to help fish to regain the ability to resume swimming. This stage of the tagging procedure is also the last opportunity to undertake a final evaluation of the individual after surgery for both predicted and unforeseen effects, and to rapidly and effectively address any issues. This may still include euthanasia, if the individual is assessed not to be suitable for immediate release. We recommend whenever possible the use of a removable V-cradle front allowing the possibility to move the fish to the side of the boat without handling it and slide the fish into the water head-first.

### Euthanasia

In the unlikely event that an adverse impact during surgery requires euthanasia as an endpoint, employment of humane methods is a *recommended practice*. This includes applying methods and procedures that are appropriate for the species (e.g., pithing), an individual's age, and development stage. The method of euthanasia must also (1) avoid pain or distress; (2) produce a rapid loss of consciousness until death occurs; (3) require a minimum restraint of the animal; and (4) ensure that is death is confirmed unequivocally [3, 67, 68].

### Training

The development of curriculum and training standards for successful BCT implantation has been identified as an important step for ensuring fish welfare [25]. Training in surgery practices include developing problem-solving competencies to manage animal welfare challenges as they arise during the surgery, as well as the technical skills to perform the surgery itself [8, 25, 27]. Welfare competencies include the skill and diagnostic abilities to identify heightened stress levels, trauma, and to monitor vital signs. Training should also include competencies in managing vessel crew and assistants who may be inexperienced in field surgery practices (and will require instruction). *Best practices* in training include organizations specifying the minimum training and qualifications required for their employees to undertake BCT implantation. This information provides material for any necessary approval or endorsement of activities by regulatory or advisory bodies and facilitates the generation of international standards in training and practices. Maintaining surgery logbooks to document who has the relevant experience to train students and colleagues, and lead BCT activities, is also beneficial for meeting any requirements set by animal research ethics committees and regulatory or advisory bodies (see "Animal ethics approval").

### Reporting

The use of BCTs in tuna research and the development and implementation of any associated methods should be a process of continuous improvement to maximize rates of post-release survival and to minimize sub-lethal effects that will bias behavioral patterns away from those normal for the species. Reporting successes and failures is a critical step for improvement as it facilitates shared learning [92]. *Recommended practices* include the use of registries for storage on open-access and easily discovered repositories. The full description of surgery methods for peer-reviewed publications will also facilitate sharing of knowledge among practitioners. At a minimum, record keeping should be sufficient to enable institutions and animal research ethics committees to verify that the wellbeing of the tagged individuals has been prioritized and allow review and critical investigation of the cause(s) of, and responses to, unexpected adverse events as a basis for developing strategies for prevention.

### Animal ethics approval

Underlying any scientific tagging program is the premise that any use of animals for scientific purposes must be ethical, humane and responsible. In many countries, this underlying premise is formalized through government regulatory processes, often facilitated through specialized committees that evaluate projects interacting with animals to ensure that they meet legislatory requirements and meet the highest possible standards when handling, using and caring for animals. In many cases, these committees provide the formal approval required (under relevant legislation) for projects to be able to utilize animals in their projects. The composition of committees varies depending on the country, but frequently they comprise evaluators from a range of disciplines and backgrounds to ensure that projects are evaluated from the perspective of the whole community and therefore meet broad ethical and social expectations. In association, many countries have developed a series of governing principles to guide the actions of those using animals for scientific purposes and it is these principles that projects are evaluated against.

**Table 1** Numbers of BCTs deployed in tropical tuna species in the western and central Pacific Ocean included in this review

| BCT type | Bigeye tuna | Skipjack tuna | Yellowfin tuna | Total |
|---|---|---|---|---|
| Acoustic tag | 240 | 133 | 330 | 796 |
| Archival tag | 1350 | 208 | 841 | 2399 |
| Total | 1590 | 341 | 1171 | 3195 |

## Assessment of recommended practices

The procedures that underpin the recommended practice approaches we describe have been developed from the combined experience of 3195 BCT surgeries performed during the implementation of tagging experiments in the Western and Central Pacific Ocean (Table 1). The most recent of these, the Pacific Tuna Tagging Programme (PTTP) [55], has tagged and released over 2780 tropical tunas with BCTs and over 463,500 individuals with external plastic identification tags since its commencement in 2006. We used data from recoveries of archival BCTs from these experiments to investigate the potential impacts of implantation on individual behavior and long-term survival, alongside informal communication and observations from the authors' network to assess practices.

Direct observations on the basis of direct and photographic observations from nine tagged tuna recaptured three to 90 days after surgery are listed in Table 2. Two tuna recaptured after three days at liberty showed evidence of partial healing of incisions. Surgical incisions of 5 tuna recaptured 6 to 11 days after release were observed to have healed closed. Tuna recaptured after 35 to 90 days ($N=2$) showed complete healing with all sutures no longer present. All fish recaptured were caught during fishing operations that required individuals to actively pursue bait and lures, indicating that normal behavior

**Table 2** Direct observation of tagged tuna recaptured from 3 to 90 days after surgery

| Tagging program | Species | Length (cm) | Tag type | Deployment duration (days) | Year | Comment |
|---|---|---|---|---|---|---|
| HTTP | YFT | 70 | Vemco V16 | 3 days | 2003 | Incision partially healed; sutures partially shed |
| HTTP | YFT | 65 | Vemco V16 | 3 days | 2003 | Incision healed closed; sutures half grown out |
| HTTP | YFT | 82 | Vemco V16 + MK9 | 6 days | 2003 | Incision healed closed; sutures almost shed |
| HTTP | YFT | 58 | Vemco V16 | 9 days | 2001 | Incision healed over, discolored |
| HTTP | BET | 58 | Vemco V16 | 9 days | 2003 | Incision healed over, discolored |
| PTTP | YFT | 55 | Lotek LAT2810 | 9 days | 2016 | Incision healed, suture loss |
| HTTP | YFT | 64 | Vemco V9 + MK9 | 11 days | 2007 | Healed closed, stitches still present |
| HTTP | YFT | 91 | Vemco V16 | 35 days | 2001 | Incision healed over, discolored |
| HTTP | YFT | 76 | Vemco V16 | 90 days | 2002 | Incision completely healed, smooth |

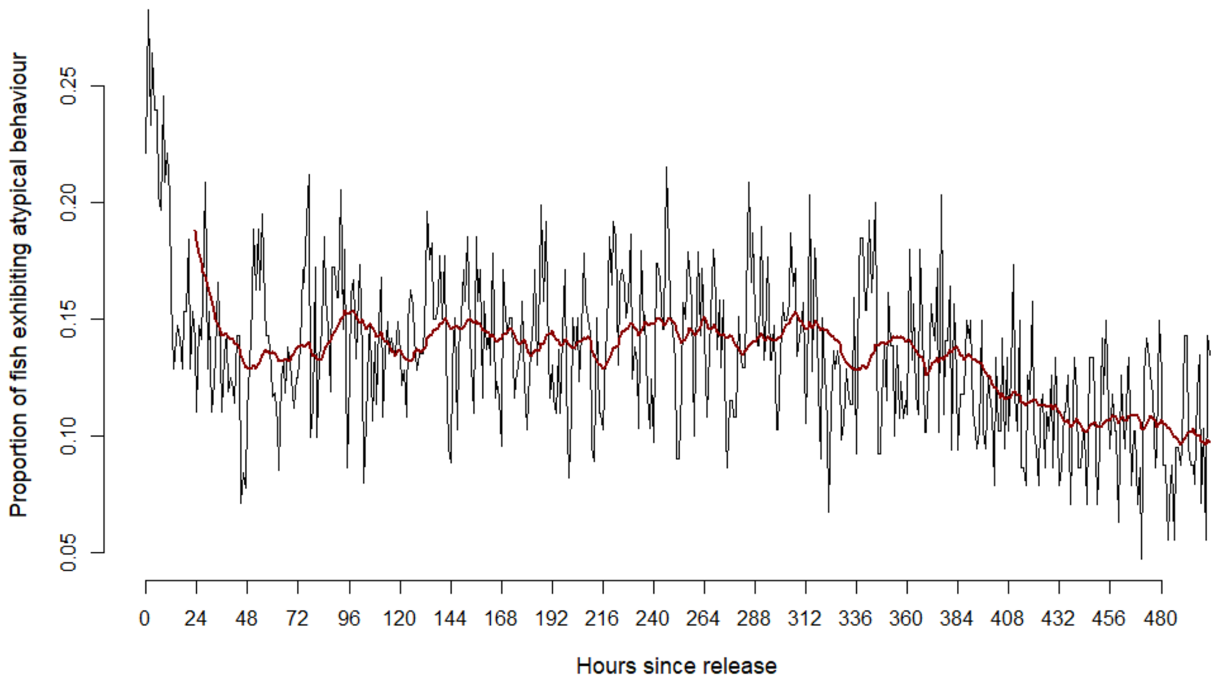


**Fig. 10** Proportion of fish exhibiting behaviors outside a 95% confidence limit of either their typical shallow or deep behaviors, as estimated by a multivariate gaussian hidden Markov model. A rolling 12-h mean is overlaid in red

had resumed rapidly after the surgical procedure and implantation of the tag.

### Comparison between returns of plastic dart tags and electronic BCTs

A small, but statistically significant, difference in returns was detected when comparing the proportional return rates of external plastic dart tags and electronic BCTs released as part of PTTP operations. In this analysis, tagging events were only considered if more than 20 individuals were released during the same event. Also, within each tagging event, any individuals that were double tagged with both sonic and archival BCTs were removed to ensure the comparison was directly between proportional returns of fish carrying single BCTs and plastic identification tags. After this filtering step, only data collected on bigeye and yellowfin tunas were included. The final dataset comprised 7339 bigeye (924 BCT, and 6415 conventional), and 4922 yellowfin (489 BCT, 4433 conventional) tuna releases. The impact of fish length was controlled for within each tagging event by omitting any fish released with external plastic dart tags whose length was smaller than the smallest fish release with a BCT. A generalized linear random effects model was fitted to the data, with a binomial response variable (where 0 = tag not recovered; 1 = tag recovered) and two explanatory variables: tag type, either plastic tag or archival BCT, and the length of the fish. A random effect to account for within tagging events was used. Modeling was undertaken in R (v. 4.1.2, [77]) using the 'lme4' package (v. 1.1-30, [5]). When fish length and tagging event is accounted for, a tagged fish was 1.35 (95% CI 1.12–1.64) times more likely to be returned if tagged with an external plastic dart tag, compared to a BCT (see Supplementary Material for a full description of the methods and results).

### Behavior analyses immediately after surgery

By identifying the presence of atypical, stress-induced behaviors during the time following release, it is possible to quantify the degree to which behavior immediately after BCT implantation is extraneous to the individual's typical behavior. To demonstrate this, the vertical movement behavior from 57 yellowfin to 106 bigeye tunas tagged and released with BCTs in the western central Pacific Ocean, and at liberty for at least one month, were examined for atypical behavior (see Supplementary Material for further details on release information). Estimating the parameters of a two-state hidden-Markov models [87, 88], the data were processed into individual time-series of hourly temperature and vertical movement frequency metrics. This approach provides each individual's shallow and deep state parameters, characteristic of typical yellowfin or bigeye tunas diurnally switching between these behavioral states [32, 59, 80]. Model parameter estimates included the multivariate gaussian mean and covariance matrices in these two dimensions, which describe the

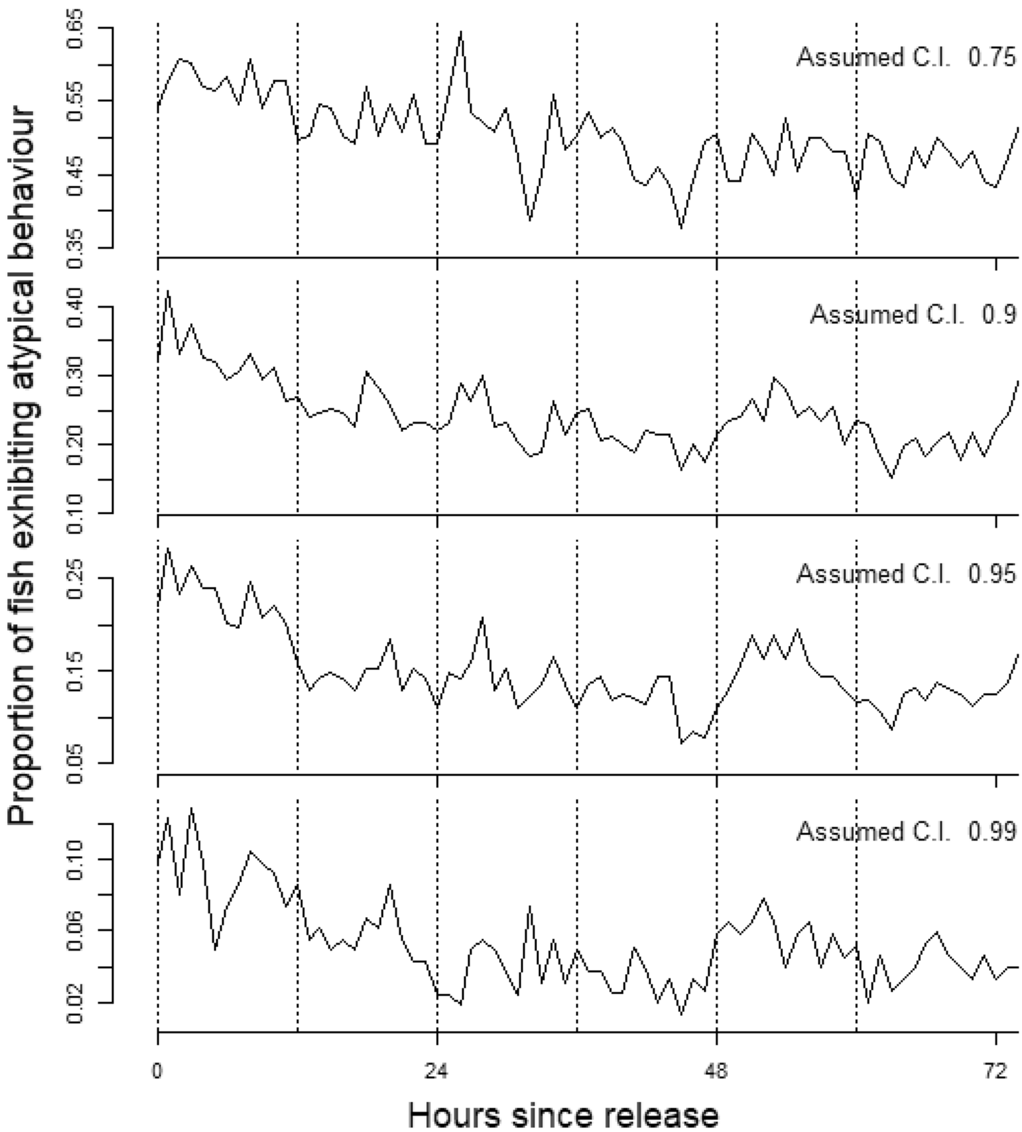


**Fig. 11** Proportion of fish exhibiting behaviors outside varying confidence limits, from 75 to 99% of either their typical shallow or deep behaviors, during the first 72 h at liberty

center and variability in water temperature occupied, and frequency of vertical movement, of these two behavioral states exhibited during the entirety of an individual fish's time at liberty.

By comparing the behavioral signal present in the hours following BCT implantation and release, to those classified over the entire time-series, we quantified the change in proportion of atypical behaviors present. Each hourly observation of water temperature and vertical movement frequency was compared to the distributions estimated for the fish's typical shallow and deep behaviors. By calculating the Chi-squared coefficient for that observation, we tested if the hourly observation lay outside a confidence ellipsoid of the estimated distributions for either of its two typical behaviors (i.e., an indication of "unusual behaviors").

Calculating this proportion of all tunas examined, approximately 25% of all individuals demonstrated behaviors differing to the rest of the behavioral time series at some point during their initial 12 h at liberty (Fig. 10). This was an increase of 13% in the mean proportion of atypical behaviors compared to when the whole time series is considered (i.e., at any time an average 12% of fish behave atypically when assuming a 95% confidence limit as normal behavior).

**Table 3** Potential standards for intracoelomic BCT implantation

| Minimum standards for the care and management of wild-caught tuna used for BCT implantation |
| --- |
| 1. General requirements that apply to all procedures must:<br>i. Be appropriate for the species and the circumstances<br>ii. Be compatible with the purpose and aims of the project or activity<br>iii. Cause the least harm, including pain and distress, to the animals<br>iv. Be performed competently, and by a person who is competent for the procedures, or under the direct supervision of a person who is competent to perform the procedures |
| 2. Fish wellbeing must be supported and safeguarded by using capture methods, techniques and equipment that are appropriate for the species and the situation, and the purpose and aims of the project or activity |
| 3. To minimize the risk of injury or stress-induced disease, procedures for the capture and handling must include:<br>i. The involvement of a sufficient number of competent people to restrain animals and prevent injury to animals and handlers<br>ii. Restraint and handling of animals for the minimum time needed to achieve the BCT implantation |
| *Release of wild-caught ensure that:*<br>4. Release occurs at the site of capture to the extent practicable,<br>5. Animals are protected from injury and predation at the time of their release,<br>6. Animals that have been sedated or anesthetized have recovered to full consciousness before their release. During their recovery, animals should be held in an appropriate area where they can maintain normal body temperature and are protected from injury and predation |
| *BCT devices*<br>7. the weight, design and positioning of attached devices must minimize interference with the normal survival requirements of the animal |
| *Humane killing*<br>8. The method and procedures used for killing (if necessary) must be humane and:<br>i. Avoid pain or distress and produce rapid loss of consciousness until death occurs<br>ii. Be appropriate to the species, age, developmental stage and health of the animal<br>iii. Require minimum restraint of the animal<br>iv. Be reliable, reproducible and irreversible<br>v. Ensure that death is established before disposal of the animal |
| *Use of anesthetics, analgesics and sedatives*<br>9. Consider the duration of the proposed procedures and balance the potential the increase in total time in distress (i.e., total handling time) associated with the use of chemical anesthesia versus the infliction of pain in fish from application of alternative methods such tonic immobility<br>10. When anesthetics, analgesics and sedatives are used, the choice of agent and its administration must:<br>i. Be appropriate for the species, age, developmental stage and physiological status of the animal<br>ii. Be compatible with the purpose and aims of the project or activity, and appropriate for the type of procedure<br>11. Regardless of their mechanism of action, the effectiveness of all anesthetics must be monitored throughout anesthesia<br>12. When general anesthesia is used, procedures must conform with current veterinary or medical practice and ensure that:<br>i. Induction is smooth, with minimum distress to the animal<br>ii. The animal and the effectiveness of the anesthetic are monitored to maintain an adequate plane of anesthesia, minimize physiological disturbances, and monitor and manage potential complications (e.g., cardiovascular and respiratory depression)<br>iii. When an animal is to recover from an anesthetic, the animal is monitored and cared for to avoid and manage complications during the post-anesthetic period (e.g., injury from uncoordinated movements or other animals)<br>iv. Records are maintained of the use of anesthetics and other drugs, monitoring of the animal, and the management of complications<br>13. Animals that develop signs of pain and distress must be treated promptly, in accordance with the intervention points and humane endpoints |
| *Surgical procedures*<br>14. The wellbeing of wild-caught tuna that have undergone surgical procedures must be supported and safeguarded by:<br>i. Using aseptic procedures if the animal is expected to recover from surgery<br>ii. Ensuring that all procedures conform to accepted standards in veterinary or medical practice, as appropriate for the procedure and circumstances<br>iii. Ensuring that potential complications during and after the procedure are avoided or minimized, that animals are monitored for complications, and that any complications that do occur are effectively managed<br>iv. Ensuring that pain management that is appropriate for the species and the procedure is effective<br>15. After any procedure:<br>i. To the extent possible fish should be monitored and assessed with sufficient frequency to ensure that both predicted and unforeseen consequences are detected early<br>ii. Prompt action must be taken so that predicted and unforeseen consequences, including pain and distress, are addressed rapidly and effectively<br>iii. Appropriate records must be maintained and made accessible to all people involved in the post-procedural care of the animal |

The analysis demonstrates that, regardless of time since BCT implantation, this consideration of tuna behaviors results in extremely variable patterns. Despite this, there appears to be an increase in unusual behaviors during the first 12 h after BCT implantation, even when confidence limits which we use to define unusual behaviors were varied (Fig. 11).

## Conclusions

We present recommended practices for BCT implantation in tunas, within the context that these will be useful for other oceanic species and with the view that these will lead to improved wellbeing outcomes for wild-caught pelagic species. In doing so this documentation provides a framework for continual refinement of practices as the experiences of practitioners develop, and ultimately help guide towards the establishment of best practices for these species. This framework can be used to describe the requirements, specifications and guidelines consistently to ensure that processes are fit for their purpose and promote best industry practices [46]. Importantly, they provide a common language to measure and evaluate performance, and ensure interoperability between practitioners [46]. As a quick reference guide, we provide an example of potential standards for intracoelomic BCT implantation in Table 3, based on the recommended practices described here. The application of these guidelines when implanting internal tags to related species can be considered and further adapted to their particular size and physiology. Where appropriate these may form a basis for the evaluation of projects by specialized committees that consider the ethical use of animals and their wellbeing in scientific research.

Our analyses indicate that the handling and surgical procedures associated with BCT insertion does have some effect on the behavior of individuals and may also affect post-release survival. However, observations from recaptured tuna suggest that these effects are small and therefore support implementation of the recommended practices described here. We believe our recommended practices help minimize the negative impacts of BCT implantation, prioritize animal wellbeing, and lead to minimal long-term impacts on tagged individuals.

#### Abbreviation

BCT Body cavity tag

#### Acknowledgements

We would like to thank the scientists, technicians and vessel crews who contributed to the BCT tagging of tuna onboard numerous research cruises.

#### Author contributions

BL and DI conceived the manuscript. SN and BL drafted the first version. JSP and JP completed data analyses. All authors technically reviewed the recommended practices and contributed to the final drafting on the manuscript. All authors read and approved the final manuscript.

#### Funding

The preparation of this manuscript was supported by the Western and Central Pacific Fisheries Commission's Pacific Tuna Tagging Programme, the Government of Australia, the Government of New Zealand, and the European Union's Pacific-European-Union-Marine-Partnership project (agreement FED/2018/397-941). This publication was produced with the financial support of the European Union and Sweden. Its contents are the sole responsibility of the authors and do not necessarily reflect the views of the European Union and Sweden.

#### Data availability

Datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

### Declarations

#### Competing interests

The authors declare that they have no competing interests.

Received: 29 August 2022 Accepted: 5 January 2023
Published online: 27 January 2023

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