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Anaesthesia of laboratory, aquaculture and ornamental fish: Proceedings of the first LASA-FVS Symposium

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Abstract

Following on from the Annual Fish Veterinary Society Conference, this symposium was organised with the Laboratory Animal Science Association and brought together experts from ornamental (pond and aquarium) fish practice, aquaculture and aquatic-research facilities to discuss good practice of anaesthesia. This proceedings paper gives an overview of relevant experiences involving a range of immersion drugs including tricaine, benzocaine and isoeugenol, as well as a summary of the main topics of discussion. While fish anaesthesia is commonplace, administration methods, drugs and monitoring procedures may often be regarded as antiquated when compared with mammalian practice. These limitations notwithstanding, individual fish will benefit from good anaesthetic monitoring. Although the most common anaesthetic drugs may be perceived as equally efficacious and therefore interchangeable for different settings, challenges are different for the anaesthesia of grouped fish, when determining species-dependent anaesthetic dosing in a multi-species tank, or adapting to farming requirements, nationally licensed products, costs and withdrawal periods. The fish anaesthetic arsenal fails to address premedication, analgesia and issues of averseness. The two latter factors should be part of the evaluation of anaesthetic protocols; therefore, instructions for the analgesic provision of lidocaine to fin clipped zebrafish are proposed. Euthanasia practices could sometimes be refined too. Alternative physical methods such as electrical stunning are options to be considered.

Keywords

Fish, anaesthesia, euthanasia, analgesia, electrical stunning, zebrafish

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Introduction

In 2017, over 1.2 million 'animals used for the first time (naïve animals) for research and testing' in the European Union were fishes.[1] The group of species constitutes the second most widely used category, after mice. Zebrafish represent 41% of the category.[1] A variety of species in wild or captive conditions complete the classification. For comparison, the output of European aquaculture in 2017 was 3 million tonnes.[2] Between 4 and 5 million UK households own an aquarium with pet fishes,[3] and while there are no estimates for numbers of private ponds and basins with ornamental fishes such as koi or goldfish, there are >70

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British public display aquaria with marine species alone, housing up to 4000 fish each.[4] These numbers suggest that fishes are used in a variety of contexts with differing needs for anaesthesia.

With all animals protected under the Directive 2010/63/EU, there is a default to using anaesthesia for any regulated procedure unless the anaesthetic procedure itself either confounds the scientific objectives or is deemed more traumatic than the condition it is meant to alleviate. By contrast, the use of anaesthetics for fish destined for the human food chain is restricted by withdrawal periods (degree-days (d/d), calculated by multiplying mean daily water temperature by total number of days since administration; for any off-label use 500 d/d is the minimum withdrawal period established by European Directive 2001/82/EC).

In order to build bridges between the laboratory, ornamental and aquaculture sectors, the Fish Veterinary Society (FVS) and the Laboratory Animal Science Association (LASA), two UK-based organisations, set up a one-day symposium with the title 'All you ever wanted to know about fish anaesthesia but were afraid to ask'. The aim was to promote the sharing of experience, by facilitating extensive discussions, and by inviting speakers to introduce current practices and challenges in fish anaesthesia. The presentations and ensuing discussions are summarised in this paper, which commences with a general overview of anaesthesia adapted to fish in comparison with mammalian procedures. Then, examples of individual anaesthesia care and monitoring for large laboratory or ornamental fish surgeries are given, followed by a case report of species-dependent anaesthetic dosing in a multi-species public aquarium tank. The next three contributions were from aquaculture, with a general introduction to routine anaesthetic procedures in farmed fish preceding a description of anaesthetic practice in Norwegian production and a summary of electrical stunning applications. The dilemma of anaesthetic averseness is then introduced, followed by an account of laboratory fish anaesthesia, with the inclusion of a usable analgesic protocol and an evaluation method of anaesthetic and euthanasic compounds. Finally, the discussion attempts to build on the wide range of fish anaesthesia experiences and contexts introduced during the symposium to address subject matters on pre-medication and anaesthetic options, analgesia and euthanasia.

Fish anaesthesia: background and definitions

Fishes, in research laboratories, farms or in a domestic or public aquarium setting, are administered anaesthetics for a wide variety of purposes, in order to reach sedation, general anaesthesia or a euthanasic overdose. Sedation, the first stage of veterinary anaesthesia, is the depression of awareness, where the animal's receptiveness to external stimuli is suppressed with arousal responses increasingly limited. Sedatives have a tranquilising and anxiolytic function, and some possess analgesic efficacy; all of which predestine them for use in anaesthetic premedication.[5] Sedation is usually recommended before handling, brief out-of-water examination or transport of laboratory, ornamental ('pet') or aquaculture fish.[6] It helps with the clinical assessment and treatment of fin or gill damage and ulcerations. Swabs, scale and gill biopsies also necessitate at the very least sedation if not anaesthesia.[7] Further uses include egg and sperm stripping of trout and salmon to supply hatcheries – in all cases withdrawal periods apply.

General anaesthesia is an obligatory intervention before invasive procedures such as tagging, cardiac excision surgery, fin clipping or amputation, as well as liver and ovary biopsy. For ornamental fish there is a whole range of invasive curative interventions which necessitate anaesthesia, removal of tumours appears to feature most commonly.[8] In aquaculture, a typical invasive purpose requiring general anaesthesia is the partial ovariectomy central to the process of 'sustainable no-kill caviar' production, although this process has been superseded by non-surgical techniques.[9]

Although overdose of anaesthesia is often the recommended method for euthanasia of ornamental fish, it is not the common practice for fish destined for the food chain: drug-free slaughter is preferred in order to avoid chemical residue. For laboratory animals, euthanasia usually follows when an animal has come to the end of its procedure. As per annex IV of the Directive 2010/63/EU, there are a number of humane killing methods which do not require licences, except for competency records for personnel who employ these methods. For fish, 'concussion/percussive blow to the head', 'electrical stunning' and 'anaesthetic overdose' are permissible, all completed with any of the legal methods listed. Physical methods are therefore deemed suitable alternatives to chemicals. Similarly, hypothermic shock is a method that triggers debate in the zebrafish community.[10–11] The discussion further elaborates on refinements of euthanasia, including the high degree of fluidity between 'anaesthetic dose' and 'anaesthetic overdose'.

Overreliance on tricaine

There is justified concern that, in the comparatively niche field of fish anaesthesia, new developments and palpable improvements are slow to take hold. In terms of qualitative changes to mainstream anaesthetic

practice, the use of tricaine (also called tricaine mesylate, tricaine methanesulfonate or MS 222) was first described for lake trout in 1960.[12] Then, tricaine and quinaldine sulfate were used in approximately equal measures;[13] arguably the main change in the ensuing 60 years was that the latter has all but disappeared from our shelves. While any perceived lack of progression and tricaine monoculture may be specific to laboratory fish, there is no published evidence that refinement and consideration afforded to anaesthesia for fishes is even approaching levels comparable to mammalian practice (Table 1).[5,6,14–18] In addition, there are concerns about the dosing of tricaine, especially with published doses for recovery anaesthesia for experimental use in zebrafish cryoinjury procedures (320–1000 mg/l)[19,20] versus veterinary guidelines (100–200 mg/l).[21]

Anaesthesia of larger laboratory and ornamental fish

Whilst smaller species such as *Danio rerio*, represent a significant part of fish used in scientific research,[1] chemical restraint of larger species is essential in research areas as diverse as physiology, aquaculture and ecology. Advances in aquatic veterinary medicine have also led to a significant increase in the use of anaesthesia for diagnostics and surgery in both research and ornamental fish.

Such work encompasses a range of taxa with wide variations in anatomy and physiology, presenting challenges from obligate ram ventilators (requiring continual swimming to 'force' ventilate, such as some sharks and teleost species) to air breathers. Nevertheless, the basic principles of anaesthesia apply but require species specific adaptations in delivery.

Pre-anaesthetic assessment should involve obtaining minimum baseline clinical data such as behaviour, activity levels, physical condition and respiratory rate/depth.[22] Preparations should involve a 12–24 h starvation period to limit both risk of regurgitation (which can impact gill tissue) and excretion of nitrogenous waste.[14] Water parameters, such as temperature and pH, in anaesthetic induction and maintenance tanks should match the source water and dissolved oxygen should be maintained at a level appropriate for the species – generally aiming for 6–10 mg/l.[6]

Induction is most commonly achieved by immersion in a dissolved anaesthetic agent. A number of chemicals can be used for this purpose such as tricaine, benzocaine, 2-phenoxyethanol, eugenol, isoeugenol and metomidate with a range of species-specific dosing information available.[14,15,23,24] Anaesthesia can also be achieved using injectable agents (e.g. propofol or

Table 1. Comparison overview of main options for fish (immersion) and mammalian anaesthetics. The table is based on the listed references.

	Mammal	Fishes (immersion route)	References
Pre-medication	Anti-emetic Anticholinergic Sedatives Anaesthetic potentiators Analgesics	n/a	5
Short-acting anaesthetic	Inhalant anaesthetic Propofol, alfaxalone	Tricaine, benzocaine, 2-phenoxyethanol, isoeugenol	5, 14, 15
Longer-acting anaesthetic	α2 adrenergic receptor agonists, dissociative agents, opioids	n/a	5
Adjusting	↓↑Inhalant concentration ↑Top-up with injection	↑Top-up immersion bath with anaesthetic stock solution ↓Provision of anaesthetic-free water if using reservoir	5, 6
Monitoring	Righting reflex Spinal reflexes Ocular reflexes (corneal & palpebral) Respiration Temperature Pulse O_2; CO_2	Righting reflex Spinal reflexes (reflexes to painful stimuli) Ocular reflexes (eye-roll) Respiration (opercular beat rate) Tapping reflex (Mauthner-initiated startle response)	5, 16, 17
Reversal	Opioids α2 adrenergic receptor antagonists	n/a 'gill flushing' to speed up recovery	5, 18

medetomidine/ketamine combinations), although results can be inconsistent, with significant species variation.[14,15,24] Oral delivery is reported but less explored.[25]

Out-of-water procedures, and ideally in-water procedures lasting longer than several minutes, should involve additional gill ventilation. This can be achieved via orally placed rubber tubing delivering normograde flow (i.e. from mouth to tail direction) of anaesthetic-laden water over the gills at an approximate rate of 1–3 l/kg/min (see Figure 1).[26] A recirculation anaesthetic delivery system is frequently used to perform surgical procedures in fish.[27]

Anaesthetic depth can be assessed via a combination of muscle tone, respiratory rate/depth, response to stimuli and perfusion indicators (e.g. gill colour). Heart rate can be monitored by Doppler flow probes or cardiac ultrasonography.[14] Electrocardiography is feasible but less routinely used. Repeat blood sampling for ancillary data such as blood pH and lactate during procedures can further inform.[24]

Recovery is achieved by placing the fish in anaesthetic-free aerated water, ensuring normograde water flow over the gills; a pump and tubing can facilitate this if strong spontaneous respiration is not present. This utilises the buccal flow–heart rate reflex whereby water flow through the buccal cavity accelerates heart rate and normalises hypoventilation-related bradycardia.[23] Recovery is typically within 5–10 minutes. Reversal agents for injectables such as α2-agonists are available. Emergency drugs such as doxopram, adrenaline and dexamethasone can be used in fish although their effects amongst the varying taxa are not well understood.[28]

Figure 1. Anaesthetised Atlantic lumpfish (*Cyclopterus lumpus*) undergoing laparoscopy. The gills are ventilated with anaesthetic-laden water via an orally placed tube.

Sedation of a multi-species tank in a public aquarium – species variation

The following is an account based on the personal observations by an aquatic veterinarian (MM) facing a variety of technical and logistical challenges in the very specialised setting of a public display aquarium. A public aquarium requested assistance with the removal of several animals from their Tanganyika display tank with a total volume of 18,000 l. The public had commented that the display was overcrowded, although this may have been largely subjective as the filtration system was able to maintain water quality and densities were not anywhere near aquaculture thresholds. In addition, a percentage of the fish showed deformities, suggestive of *Mycobacterium* infections and inbreeding defects, amongst other causes.

Integrity of viewing windows in older displays is usually held in place by water pressure. Draining the aquarium had been carried out on previous occasions. Due to the age of the installation, this could not be done anymore as it would have caused window sealing issues. Netting was also not possible due to the display reef structure. Electrofishing was discussed, but as the display was bordered by sharks, which are considered sensitive to electric fields, with only a concrete wall separating the two, this was dismissed. Rod and line fishing was thought to target only the more able fish. Chilling the aquarium could technically not be achieved.

This left anaesthesia by immersion as the only option. Aqui-S (isoeugenol, Aqui-S vet., Scanvacc AS, Hvam, Norway) was not available in this particular country while 2-phenoxyethanol was not on the facility's discharge consent. The only two commercial aquaculture options were tricaine and benzocaine. The former was found too expensive, therefore benzocaine, brought into solution with chemical grade ethanol, was used.

Reported doses for benzocaine sedation depend on fish species and vary from 25 to 200 mg/l.[15] In absence of data for the three hosted cichlid species, *Neolamprologus brichardi*, *Lobochilotes labiatus* and *Oreochromis Tanganyika*, the anaesthetic was added in 12.5 mg/l increments. Doses required to reach sedation allowing netting and removal varied significantly between species: 25 mg/l of benzocaine was enough to sedate *N. brichardi*; *L. labiatus* sedation required 50 mg/l; and sedation of *O. Tanganyika* was only achieved with 87 mg/l of benzocaine.

Once all surplus and deformed animals were caught, they were euthanized with an overdose of benzocaine (> 300 mg/l). When flushing started, pockets of anaesthetic remaining at the bottom were noticed, they were

possibly due to the solution's higher density or the lack of circulation. Some fish had been overdosed in these pockets, and their cadavers were removed promptly. In the days following the removal, the display also experienced a substantial bacterial bloom believed to be caused by the carbon from the added alcohol.

Anaesthesia in farmed fish

In fish farming, depending on the market, several products are available to perform chemical anaesthesia. These products are dissolved in water as a powder or liquid form, taken up through fishes' gills whilst in immersion, and excreted via gills and urine.[15] General anaesthesia is a reversible process which comprises six stages, each affected with a fair degree of dose-dependency.[29] According to the author's (JN) procedural experience, four stages of anaesthetic recovery are observed once fish are returned to non-medicated water. First, operculum movement reappears. Then equilibrium and swimming motion are partially recovered. Full equilibrium recovery marks the third stage, before final total behavioural recovery with normal swimming.

The level of anaesthesia depends on the anaesthetic itself, dose, water temperature, immersion time, biomass, fish species, size and type of handling. The ideal anaesthetic will induce rapid anaesthesia with quick excretion and recovery, a wide margin of safety and a short or absent withdrawal period.[15] Anaesthesia is required for several procedures on farmed fish such as vaccination, harvest, transport, clinical procedures and euthanasia, ensuring loss of pain, stress and immobilisation, the latter also improving operator safety.[30] Immobilization of farmed fish is especially important for vaccination, both for welfare reasons and operator safety, to avoid self-injections. This is done for hand and machine vaccination. The vaccine needs to be injected intracoelomically between the anus and the pelvic fins. This avoids unwanted effects resulting from misplacing the deposit of vaccine and ensures a correct immune response.[31,32]

Fish sedation and anaesthesia in Norwegian aquaculture

In Norway, four molecules are licensed for fish sedation and anaesthesia: benzocaine (Benzoak vet., ACD Pharmaceuticals AS, Oslo, Norway), isoeugenol (Aqui-S vet., Scanvacc AS, Hvam, Norway), metomidate (Aquacalm vet., Scanvacc AS, Hvam, Norway) and tricaine (Finquel vet., Scanvacc AS, Hvam, Norway; Tricaine Pharmaq, PHARMAQ, Overhalla, Norway). These are mainly used in salmon, for which there is a national annual production of 1.2 million tonnes (i.e. 240 million individual fish at 5 kg harvesting weight), worth over 3.5 billion euros.[33]

Sedation is administered during stressful handling, typically when collecting and pumping fish for smolt delivery and transport, bath treatment in well boats or pens, brood stock sorting as well as anaesthetic premedication (LS personal observation).

In 2016, isoeugenol was used for sedation on approximately 100 million fish which would have been fit for human consumption after a withdrawal period of 2 d/d.[34] The molecule is derived from clove oil, naturally occurring in food, used either as a feed additive or, as eugenol, as a local anaesthetic in human dentistry. The onset time to efficacy with 2.5 mg/l isoeugenol is about 5–15 min, at which point fish swimming activity, oxygen consumption, carbon dioxide production as well as anxiety, are reduced.[15] Salmon do not lose swimming ability or equilibrium at this dose and cortisol levels will show a minor rise following sedation *per se*. The cortisol rise caused by subsequent handling will however be significantly reduced compared to non-sedated fish.[35] Isoeugenol reduces cortisol rise by intercepting stressor perception in the hypothalamus–pituitary–interrenal axis.[24] This is to be differentiated from anaesthetising dose rates. At 12.5 mg/l, salmon are anaesthetised within 15 min and cortisol levels rise. At 50 mg/l, isoeugenol can induce death without significant increase of cortisol level.[36]

Anaesthesia is indicated for precision work requiring fish immobilization, such as vaccination (about 350 million fish per year in Norway), sea lice counting (about 1.25 million fish per year in Norway) or brood stock tagging and sampling.

Tricaine and benzocaine are the main anaesthetics used. Tricaine is licensed for doses up to 135 mg/l.[37] For vaccination, it is often used at 350 mg/l to achieve a 45 s induction and avoid significant cortisol rise. Tricaine shows poor stress reducing capacities when used as a sedative. Benzocaine is licensed for up to 40 mg/l,[38] and seems to induce higher mortalities at high temperatures (>15°C for salmonids).[15] Metomidate is not licensed for fish destined for human consumption,[39] and its use remains dedicated to research. Metomidate blocks the cortisol synthesis in the interrenal cells.[40] At the recommended dose of 5 mg/l, induction and recovery are fast.[15]

Electrical stunning

Applications of electricity in fisheries include electrofishing,[41] electric deterrence systems for fish[42] and electrical stunning (humane slaughter).[43] Electro-anaesthesia was explored in a range of species, including humans, in the 20th century, before waning due to the significant advances with chemical anaesthesia.

Nevertheless, electrical stimulation of neural tissue is still used within modern medical practice.[44]

Technical reports on the welfare of farmed fish at the time of slaughter recommend electrical stunning, when performed adequately (e.g. *Advancing aquaculture: Fish welfare at slaughter*,[45] StunFishFirst final report,[43] European Food Safety Authority,[46] Farm Animal Welfare Committee[47] and Royal Society for the Prevention of Cruelty to Animals[48]). In the aquaculture industry, the target is 100% successful fish stunning with perfect carcass quality. Thus, industrial output aims for a reliable and injury-free killing method. The electric field must be controlled to avoid haemorrhages or spinal fracture, i.e. welfare must be optimized. The electrical stun is designed to render fish unconscious within one second, and to prevent recovery during the slaughter process.[49]

The electrical stun efficacy depends on several parameters: fish species, water conductivity, electrode array configuration, current waveform and voltage. Electrical stunning of farmed fish is possible within a water conductivity range of about 50–50,000 μS/cm (i.e. very low conductivity freshwater to full strength seawater).[41] Continuous in-water electrical stunning systems typically deploy electric fields inside pipelines suitable for the fish size range, while batch in-water electrical stunning systems use circular tanks with a customised electrode array (MOF personal observation).

Aversive properties of anaesthetic drugs

As with all other common veterinary drugs, anaesthetic agents are routinely used for fishes, for surgical and husbandry procedures. However, anaesthesia protocols for fishes are often historic and usually refer to a single agent; there are very few specific protocols when accounting for the 32,000+ species.[50] As summarised by Readman et al. (2017),[51] several studies using different behavioural assessment methods have highlighted that the use of anaesthesia in fish is far more complicated than originally thought. Aversion to a particular anaesthetic in one species does not necessarily equate to aversion in another.

Across Europe, the standard anaesthetic for fish is tricaine.[52] In order to assess the potential aversion of four commonly used laboratory species – medaka (*Oryzias latipes*), carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*) and fathead minnows (*Pimephales promelas*) – to three anaesthetics – tricaine, benzocaine and etomidate – a chemotaxic test chamber was used following the methods previously used by Readman et al. (2013)[50] to test the aversion of zebrafish (*D. rerio*) to the same anaesthetics.

All of the tests showed differences in reaction to the test anaesthetics. Tricaine and benzocaine induced avoidance behaviours in medaka and this response was similar to that of zebrafish previously reported by Readman et al. (2013).[50] Carp, as opposed to zebrafish and medaka, showed avoidance behaviours to etomidate, but displayed no aversion to benzocaine or tricaine. Rainbow trout showed no aversion to any of the three agents tested. Of great interest was the fact that it was not possible to illicit an avoidance response from fathead minnows to any of the anaesthetics or in fact the positive controls. The fathead minnow is a recommended species for many regulatory testing protocols (e.g. Organisation for Economic Co-operation and Development (OECD), United States Environmental Protection Agency (US EPA)), and it has been used for preference and avoidance testing for aquatic contaminants.[53–55] Information presented by Readman et al. (2017), however, suggests that this species is not suitable for such models due to the inability to produce a behavioural response.[51]

European Directive 2010/63/EU prescribes that regulated procedures should not be conducted without anaesthetic, and that for all procedures the use of anaesthesia should have a benefit aligned with the aims of the procedure. There is limited information on the effectiveness of several anaesthetics used with fish and further work is needed within this field to understand the behavioural response to anaesthetics in a greater number of species, also accounting for any strain differences within individual species. It is therefore paramount that work undertaken with fish species involving any element of anaesthesia must be reviewed to ensure that best practice is used commensurate with the aims of the study and that this is done on a species-by-species basis.

For example, zebrafish and carp are both cyprinids and yet display differing responses (e.g. dose to effect, aversion) to anaesthetics.[50,51] Consideration must therefore be given to the morphology and behaviour of individual species, as well as the specific aims of the study, and any resulting evidence-based protocol change should be adopted as good practice for fish anaesthesia.

Fish anaesthesia in the laboratory

Anaesthesia protocols applied to research fish take in account events preceding the scientific procedure and fish fate. For example, animals just caught in the wild by trawling or electrofishing are under physiological stress, and reduced dose rates suffice to induce surgical anaesthesia (JPM personal observation). If fish are then released back to the wild, post-operative care is compromised: analgesia, pain and adverse effects are

difficult to control. Moreover, when performing surgery, imaging or other prolonged scientific procedures requiring immobilisation, a single animal is anaesthetised and closely monitored.[56] By contrast, for fast and routine procedures like gamete collection or fin clipping of *D. rerio*, anaesthesia or heavy sedation is induced and not maintained.

Tricaine is often the preferred anaesthetic in this context since anaesthetic depth is easy to control and mortality low or reduced with pre-medication (e.g. 20% of induction dose). Nevertheless, tricaine may not be the perfect choice as repeated exposure increases the risk of mortality,[57] it is aversive,[50,58] and tricaine anaesthesia does not cover post-recovery analgesia needs following painful procedures. For the latter, lidocaine hydrochloride 2–5 mg/l is added to holding tank pre-op and to the recovery tanks[59] until the next water change (i.e. 48 h), as detailed in Table 2. This does not involve any supplementary netting. Experience on thousands of zebrafish with fin clipping and administrations show no increased mortality.

To select alternatives to tricaine, anaesthetics can be reviewed, through literature and personal experience, and scored according to what would constitute the perfect anaesthetic, for example mistake proof preparation, fast induction and recovery, reliability reaching required anaesthesia depth, low mortality and delayed toxicity, analgesic, low averseness, safe for personnel and environment. For example, such a theoretical compound screening system is applied in the context of fast and routine procedures for laboratory zebrafish, with the following candidates: benzocaine, lidocaine, tricaine, etomidate, metomidate, 2-phenoxyethanol, clove oil and isoeugenol. In the absence of data on anaesthetic potency, isoeugenol receives the best score and is therefore selected to be trialled. Similarly, application of the theoretical compound screening system to the scoring of anaesthetic reliability to achieve euthanasia by overdose – ease of preparation and ability to cause death fast and permanently on all animals – leads to the non-selection of molecules such as metomidate, due to its lack of euthanasic potency,[60] and despite its lesser averseness.[58]

Discussion

The symposium took in contributions from laboratory animal and aquaculture clinicians, ornamental fish vets and industry representatives. At the outset, a lack of anaesthetic options and refinements was observed, with an overwhelming reliance on tricaine. Attendees who had not tried alternative agents stated potential regulatory issues when using off label compounds. The use of other agents should be justified by the publication of studies detailing potency and disadvantages. Suggestions for more species specific anaesthetic protocols are not new,[51,60] where focus has either been on aversive properties of a range of anaesthetic agents on various species[50,51,58] or on potency – usually measured on speed of induction and recovery, reliability in reaching required anaesthetic depth, and any interference with physiological parameters.[60,61] There is some concern that conclusions on averseness may not be

Table 2. Peri-operative analgesia protocol for zebrafish fin clipping.

1. Prepare a lidocaine stock solution (LSS) with 530 mg of lidocaine hydrochloride per litre of system water.
2. Remove the fish to be fin clipped from the recirculation system.
   (a) The fish tank is taken away from other fish sight.
   (b) The tank is set on a bench that is not of a colour contrasting with the system shelves, or the tank is set on a photo of gravel.
3. Add some LSS to reach 2–4 mg/l lidocaine in the holding tank.
   (a) For a tank of 3 l, you can use four 3 ml pipettes of LSS.
4. Prepare all equipment while fish swim in the analgesic solution in their home tank and absorb the analgesic.
   (a) Thaw and/or mix anaesthetic solution.
   (b) Fill up and label recovery tanks.
5. Add LSS to each recovery tank to reach 2–4 mg/l lidocaine.
   (a) When breeding tanks are used as recovery tanks, they contain 400–800 ml of system water. Pour one 3 ml pipette of LSS into each recovery tank.
6. Anaesthetise and fin clip fish as usual.
   (a) A solution of tricaine (168 mg/l) can be used for anaesthesia, as long as it is buffered in tris or with 336 mg/l sodium bicarbonate in system water.
7. As soon as a fish is fin clipped, transfer it to a recovery tank medicated with 2–4 mg/l lidocaine.
   (a) A maximum of two fish are kept in a recovery tank with 400–800 ml system water.
   (b) Fishes in recovery tanks are fed once a day in reduced quantity.
   (c) Recovery tanks are set on a bench that is not of a colour contrasting with the system shelves, or the recovery tanks are set on a photo of gravel.
8. Keep fish in the recovery tank containing 2–4 mg/L lidocaine until the next water change, a maximum of 48 h later.

reconcilable with data on toxicity as least aversive compounds such as etomidate may induce mid- to long-term adverse effects.[61]

Despite a perceived monoculture of tricaine as sedative, anaesthetic and euthanising agent in research laboratories, accounts from ornamental fish anaesthesia have shown a wider range of chemicals and administrative routes. Therefore, there is scope to expand the anaesthetic arsenal for laboratory fish. To an extent, this is already the case in aquaculture where different compounds are routinely used (e.g. isoeugenol, tricaine, benzocaine). The trial deployment of isoeugenol for anaesthesia or pre-medication of smaller fish species is largely seen as promising, but limited product availability and obstacles to sourcing are an issue. There was widespread agreement across stakeholders that the prescribing of fish anaesthetics is cumbersome, and that this has been one of the reasons for the slow evolution of anaesthetic protocols. It is also to be noted that some species may require specific considerations, for example metomidate is contra-indicated in air-breathing fish.[39] Tonic immobility, a reflex state of motor inhibition, can be artificially induced in some species (e.g. elasmobranchs, sturgeon, some catfish) allowing handling for minor procedures when anaesthesia or analgesia are not required.

The refinement of anaesthetic protocols may involve the use of pre-medication before anaesthesia induction, as is routinely performed in mammals. Patients are pre-medicated to reduce the doses of induction and maintenance drugs, to optimise conditions for a smooth induction and recovery and to provide pre-emptive analgesia. In addition, veterinary pre-medication protocols strive to improve safety for personnel. Typical pre-medication protocols in veterinary practice provide moderate to profound sedation as well as analgesia, anxiolysis and muscle relaxation. Diazepam[62] and midazolam,[63] two compounds usually subject to national regulations regarding their use and supply and often prescribed for mammal pre-medication, can be used to modulate the fish stress response, although applications in the context of fish anaesthesia remain undescribed. Tricaine (Tricaine Pharmaq, PHARMAQ, Overhalla, Norway) is licensed for fish sedation at 30 mg/l. At the symposium, the consensus was that fish should benefit from anaesthetic pre-medication, and viable published protocols were needed.

However, refining anaesthetic administration itself may prove more effective than adding a sedative step. For example, bearing in mind handling stress, it should be considered on a case-by-case basis whether fish should be moved to anaesthetic baths, or whether adding anaesthetic to their home tank is a better option. Moreover, emphasis was on getting the basics right by observing acclimatization periods, providing sufficient oxygen to the anaesthetic solution, and by buffering the immersion tank to the holding system's pH.

Despite analgesia for fish having already been subject to controversial debate,[64] there was general agreement among symposium stakeholders that analgesic additions to anaesthetic protocols would constitute a refinement, with usable options (see, for example, Table 2) already published.[59,65] These protocols list examples of potentially efficacious compounds from different pharmaceutical groups, for example lidocaine, morphine and non-steroidal anti-inflammatory drugs. Participants who had provided analgesia to fish, declared having also used benzocaine, tricaine and butorphanol for that purpose. Rejection of analgesic use for potentially painful procedures has been justified through lack of evidence that procedures would induce pain, or that compounds would alleviate induced pain. This argument wears thinner with every additional piece of published evidence.

On the subject matter of euthanasia, the participants deemed that recommendations should depend on situation, species and environment. It is suggested that death occurs 'usually, but not always' when fish are maintained, for 5–10 min after cessation of opercular movement, immersed in a solution at 5–10× the anaesthetic concentration.[14] This blanket advice leaves room for improvement as the euthanasic potency of compounds may vary according to their chemical properties, the water conditions and the fish developmental stage.[14,60,66,67] In research laboratories, where large throughput of fish occurs, it is important that routine does not work to the detriment of good care. For example, conscious fish should not be dropped into anaesthetic overdose immersion baths already containing euthanized individuals.

The participants also raised the question of what would be a 'better' death: an almost instant loss of consciousness and quick death potentially fraught by a momentary aversive experience or a slow but stress-free process? Considering the aversive properties of immersive anaesthetics, and the limited potency of less aversive ones,[60] it does not seem that options for slow and stress-free methods are available yet. Therefore, methods leading to a fast death may lead to a refined process, in the right context. For example, physical methods may constitute suitable alternatives to avoid a distressing anaesthesia.[68] Concussion/percussive blow to the head followed by completion of death before the return of consciousness is an acceptable option, including for aquaculture slaughter, when stress linked with handling and out of water exposure is minimised. Similarly, electrical stunning, when carried out appropriately, may be an effective option to terminate promptly a group of fish and avoid handling

stress.[68] Equipment adapted to the laboratory context and to different sizes and developmental stages of fishes are under trial. However, questions remain unanswered regarding fish experience and potential pain intensity during induction of the electrical stun, before unconsciousness is reached. Another physical method that occupies debates on laboratory fish euthanasia is the hypothermic shock. This does not require expensive equipment, as it relies on suddenly immersing small tropical fish (i.e. *D. rerio* size) kept at $> 26°C$ into ice-chilled water ($< 4°C$). Adult fish die in a couple of minutes,[10,69] but often while displaying signs of discomfort.[11] Again, fish experience remains unexplored with this physical method. The technique is not suitable for the fast euthanasia of larvae.[10]

Finally, symposium stakeholders raised two ethical points. The first issue was the difficulty of complying with requirements on completion of euthanasia when researchers need to harvest a fresh brain rather than destroy it to confirm death (as prescribed by the regulator). The second topic was the use of sedation. In some cases, the difference between practices undertaken for the purposes of recognised animal husbandry and procedures performed for an experimental purpose, that is requiring regulation under the Directive 2010/63/EU, can be difficult to determine. Some fish husbandry procedures are routinely conducted with sedated fish, some experimental procedures may rely on the exemption afforded to routine husbandry processes, and it is not always clear how to define the threshold at which a set of protocols needs to become regulated.

Conclusion

This first LASA-FVS symposium constitutes an unprecedented cross-over between sectors which allowed detailed introductions of fish anaesthesia practices in research, ornamental and aquaculture set-ups. It triggered discussions towards refinement of practices in all contexts, and more specifically regarding premedication, analgesia, applying a diverse anaesthetic arsenal and best euthanasia practice. The next such symposium may focus on how the different sectors and stakeholders can overcome their different objectives and profit better from each other's experiences. For example, the discussions raised two regulatory conundrums: the use of sedation for husbandry procedure (e.g. tagging, transport, handling) versus regulated procedures, and the veterinary prescription and delivery of un-licensed molecules (i.e. as alternatives to licensed products) for the refinement of anaesthesia and euthanasia protocols or to provide analgesia. It could be beneficial to invite national regulatory bodies to inform these topics further, all the more in times of potential UK departure from Directive 2001/82/EC and its prescribing cascade for unauthorized medicines. Thereby stakeholders could build on an opportunity to create unprecedented synergies by reconciling criteria specific to the different sectors, such as withdrawal periods, averseness, surgical planes and out-of-water procedures, with protocols which exclusively focus on fish welfare.

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Résumé

Suite à la Conférence annuelle de la Société vétérinaire du poisson, ce symposium a été organisé avec l'Association des sciences animales de laboratoire, et a réuni des experts des installations de poisson ornemental (étang et aquarium), d'aquaculture et de recherche aquatique pour discuter des bonnes pratiques en matière d'anesthésie. Ce document fournit un aperçu des expériences pertinentes concernant une gamme de médicaments d'immersion, notamment la tricaïne, la benzocaïne et l'isoeugénol, ainsi qu'un résumé des principaux thèmes de discussion. Bien que l'anesthésie des poissons soit courante, les méthodes d'administration, les médicaments et les procédures de surveillance utilisés peuvent souvent être considérés comme obsolètes par rapport à la pratique chez les mammifères. Nonobstant ces

limitations, chaque poisson bénéficiera d'une bonne surveillance anesthésique. Bien que les médicaments anesthésiques les plus courants puissent être perçus comme tout aussi efficaces et donc interchangeables pour différents environnements, les défis sont différents pour l'anesthésie de poissons groupés, lors de la détermination de la dose anesthésique en fonction de l'espèce dans un réservoir multi-espèces, ou de l'adaptation aux exigences agricoles, aux produits homologués au niveau national, aux coûts et aux temps d'attente avant consommation. L'arsenal anesthésique du poisson ne prend pas en compte la prémédication, l'analgésie et les questions d'aversion. Les deux derniers facteurs devraient faire partie de l'évaluation des protocoles d'anesthésie; des consignes analgésiques par administration de lidocaïne aux poissons zèbres à nageoire coupée sont proposées. Les pratiques d'euthanasie pourraient également être raffinées dans certains cas. D'autres méthodes physiques comme l'étourdissement électrique sont des options à prendre en compte.

Abstract

Im Anschluss an die Jahreskonferenz der Fish Veterinary Society fand das hier besprochene Symposium in Zusammenarbeit mit der Laboratory Animal Science Association statt, bei dem Experten aus der Zierfischpraxis (Teich und Aquarium), der Aquakultur und aus Einrichtungen aquatischer Forschung zusammenkamen, um gute Anästhesiepraktiken zu diskutieren. Dieser Tagungsbericht gibt einen Überblick über einschlägige Erfahrungen mit einer Reihe von Betäubungsmedikamenten, darunter Tricain, Benzocain und Isoeugenol, sowie eine Zusammenfassung der wichtigsten Diskussionsthemen. Obwohl Anästhesie bei Fischen üblich ist, sind die Verabreichungsformen, Medikamente und Überwachungsverfahren im Vergleich zur Praxis mit Säugetieren häufig als veraltet anzusehen. Ungeachtet dieser Einschränkungen profitieren einzelne Fische von einer guten Anästhesieüberwachung. Obwohl die gebräuchlichsten Anästhesiemittel als gleich wirksam und daher in verschiedenen Szenarien als austauschbar gelten können, gibt es unterschiedliche Herausforderungen bei der Anästhesie von Fischgruppen, nämlich bei der Bestimmung der artabhängigen Anästhesiedosis in Mehr-Arten-Becken oder bei der Anpassung an Haltungsbedingungen, national zugelassenen Produkten, Kosten und Karenzzeiten. Das Arsenal der Fischanästhetika berücksichtigt nicht Prämedikation, Analgesie und Aversion. Die beiden letztgenannten Faktoren sollten jedoch Teil der Bewertung von Anästhesieprotokollen sein; daher werden Anleitungen für die Verabreichung von Lidocain als Analgetikum an flossenamputierte Zebrafische vorgelegt. Auch Euthanasie-Praktiken könnten ggf. verbessert werden. Alternative physikalische Methoden wie die elektrische Betäubung sind hier in Betracht zu ziehen.

Resumen

Después de la Conferencia Anual de la Sociedad Veterinaria de Peces, este coloquio fue organizado con la Asociación Científica de Animales de Laboratorio y reunió a expertos en peces de decoración (estanques y acuarios), acuicultura e instalaciones de investigación acuática para hablar sobre buenas prácticas de la anestesia. Este informe de procesos ofrece una perspectiva general de experiencias relevantes que conllevan una serie de medicamentos de inmersión como la tricaína, la benzocaína y el isoeugenol, así como un resumen de los principales temas del coloquio. A pesar de que la anestesia de peces es una práctica habitual, los métodos de administración, los medicamentos y los procesos de control a menudo pueden considerarse como anticuados al compararse con los utilizados con los mamíferos. A pesar de estas limitaciones, los peces individuales se beneficiarán de un buen control de los métodos de anestesia. A pesar de que los medicamentos anestesiantes más habituales pueden percibirse como igualmente eficaces y, por tanto, intercambiables para otras situaciones, los retos para la anestesia de grupos de peces son diferentes al tener que determinar dosis de anestesia específicas para cada especie en un tanque donde hay varias especies de peces o al tener que adaptar requisitos de cría, productos licenciados nacionalmente, costes y periodos de retirada. El arsenal anestésico no puede cubrir la premedicación, la analgesia y los problemas de reacciones. Los dos últimos factores deberían formar parte de la evaluación de protocolos de anestesia, por tanto, se propone instrucciones para la provisión analgésica de lidocaína para el pez cebra con aleta cortada. Asimismo, a veces también se podrían refinar las prácticas de eutanasia. Otros métodos físicos alternativos como el aturdimiento eléctrico también son opciones a considerar.