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Popular Article

Exploring Anesthetic Strategies in Aquaculture: Enhancing Welfare and Management Practices

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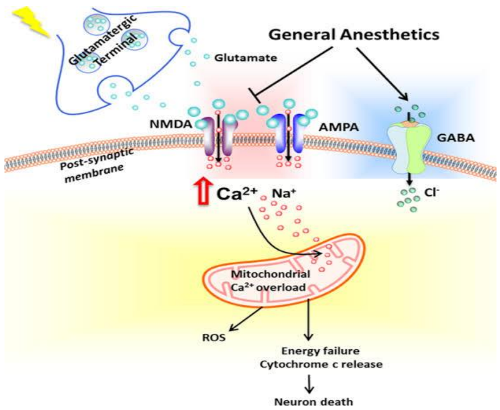
Introduction

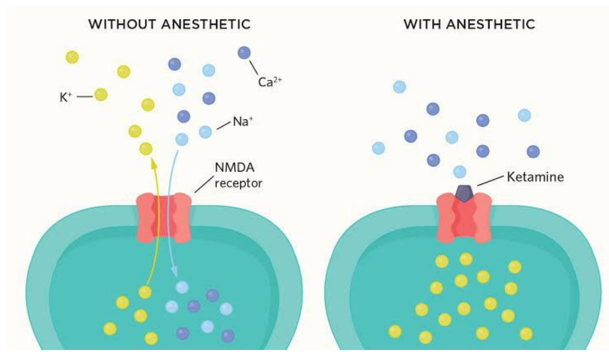
An anaesthetic serves as an agent capable of inducing either local or general loss of sensation, thereby mitigating pain. The term anaesthesia finds its roots in two Greek words, with "an" signifying "without" and "aesthesis" meaning "sensation". The integration of anaesthetics in medical and surgical practices proves pivotal, facilitating procedures without inflicting undue distress or discomfort. The inherent susceptibility of fish to stress during handling and transport underscores the necessity of anaesthetic intervention.

Stress, if unchecked, can lead to immuno-suppression, physical injury, or even mortality. In the context of aquaculture, anaesthetics find application during transportation to avert physical harm and curtail metabolism, encompassing dissolved oxygen consumption and excretion. Furthermore, these agents serve to immobilize fish, easing handling processes during harvesting, sampling, and spawning procedures.

Mode of action- Anaesthetic agents operate by interacting with membrane proteins, each exhibiting distinct molecular mechanisms. The primary sites of anaesthetic action include the thalamus and the Reticular Activating System.

In the case of local anaesthetics, their function involves impeding the initiation and propagation of action potentials through the blocking of voltage-sensitive sodium channels. The administration of anaesthetics like MS222 which enters the circulatory system via gill uptake induces general anaesthesia by inhibiting neural signal transmission ranging from the periphery to higher parts of the nervous system. Notably, anaesthetics impact adrenal steroidogenesis, suppressing cortisol production in fish. This suppression may encompass an expansion of neuronal cell membranes, thereby dampening activity in the central nervous system. The predominant mechanism of action for Tricaine Methanesulfonate (TMS) involves the suppression of the nervous system, wherein the entrance of sodium (Na) into the nerve is hindered, limiting nerve membrane excitability. TMS's ability to traverse cell membranes due to its lipid solubility enhances nerve inhibition by facilitating binding with sodium channels.





Characteristics of a good anaesthetic agent

The characteristics of an ideal anaesthetic encompass a multifaceted set of criteria. Primarily, anaesthetic should be non-inflammable and non-explosive, ensuring safety during use. Furthermore, the anaesthetic should offer sufficient analgesia, affording relief from pain. Rapid induction and recovery are paramount, accompanied by the absence of lingering after-effects. Importantly, the ideal anaesthetic should not induce nausea or vomiting, maintaining the overall well-being of the subject. Non-irritating qualities, coupled with a pleasant experience, contribute to the overall effectiveness of the anaesthetic. The administration process should be facile, controllable, and versatile, allowing for adaptability in various scenarios. Potency is a crucial attribute, requiring low concentrations for efficacy while ensuring optimal oxygenation for the subject. Additionally, the ideal anaesthetic should exhibit stability, effectiveness, and facile storage, collectively addressing the practical aspects of its application.

Commonly used anaesthetics in fish: Some of the commonly used anaesthetics and their dosages are tabulated below.

Sl.No	Anaesthetic	MS-222	Benzocaine	Quinaldine	2-Phenoxyethano	Metomidate	Clove oil	Aqui-S
1	Nile tilapia	100-200 mg/L	25-100 mg/L	25-50 mg/L	400-600 mg/L	ND	ND	ND
2	Common carp	100-250mg/L	ND	10-40mg/L	400-600 mg/L	ND	ND	ND
3	Channel catfish	50-250mg/L	ND	25-60 mg/L	ND	4-8 mg/L	100 mg/L	20-60 mg/L
4	Rainbow trout	40-60 mg/L	25-50 mg/L	ND	100-200 mg/L	5-6 mg/L	40-120 mg/L	20 mg/L
5	Carp and grasscarp	20 mg/L	ND	ND	ND	ND	ND	ND
6	silver carp	10 mg/L	40-100 mg/L	100 mg/L	40-120 mg/L	ND	25-100 mg/L	ND
7	Bighead carp	35 mg/L	ND	20-60 mg/L	20 mg/L	ND	ND	ND
8	livebearers	30mg/L	ND	ND	ND	ND	ND	ND
9	Silver perch	ND	20 mg/L	ND	ND	ND	ND	ND
	Freshwater eels	ND	60 mg/L	ND	ND	ND	ND	ND
11	Snappers	ND	ND	ND	ND	50 mg/L	ND	ND
	Sea bass	ND	ND	ND	ND	ND	6-9 mg/L	ND

MS-222

The chemical compound known as MS-222 is scientifically labelled Tricaine Methanesulfonate. As the sole chemical anaesthetic approved by the FDA for use on food fish, MS-222 necessitates a 21-day withdrawal period. Widely recognized as one of the most utilized anaesthetics for poikilotherms globally. It is commercially available under the names Tricaine-S and Finquel. This compound, presented in the form of a white, crystalline powder, effortlessly dissolves in water, yielding a solution of up to 11%, which can be further buffered with sodium bicarbonate (baking soda) to achieve a pH of 7. Notably, caution is advised against exceeding 250 mg/L for warm-water fish.

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MS-222 boasts several advantages, including rapid induction in 15 seconds. Furthermore, its swift excretion in fish urine within 24 hours and subsequent decline in tissue levels to near zero contribute to its safety profile. Another noteworthy advantage lies in its 11% solubility, forming a clear, colourless acid solution, enhancing its ease of use and application in various aquatic settings.

Nevertheless, MS-222, despite its merits, is not without its drawbacks. The mandated 21-day withdrawal period imposed by the FDA diminishes its practicality as an anaesthetic for fish destined for the market, limiting its applicability in certain contexts. Moreover, MS-222 has been associated with an increase in cortisol levels, even in the absence of physical stressors, presenting a potential concern for the well-being of fish. A significant limitation emerges as handling, even during deep anaesthesia, leads to elevated plasma cortisol concentrations, indicative of stress. Additionally, reports highlight reversible retinal toxicity linked to chronic occupational exposure, necessitating the use of protective clothing to prevent contact during its application. Furthermore, the compound's capacity to lower water pH introduces an acidic condition, posing an irritant to fish and potentially resulting in harmful side effects. Careful consideration of these disadvantages is crucial in assessing the suitability of MS-222 in various aquatic scenarios.

Benzocaine

Benzocaine, also known as ethyl aminobenzoate, emerges as a compelling alternative to MS-222, sharing chemical similarities while presenting distinct characteristics. As a white crystal, benzocaine stands out for its almost insolubility in water, necessitating prior dissolution in ethanol or acetone. Remarkably, it mirrors tricaine's effectiveness, demonstrating efficacy at doses ranging from 25 to 100 mg/L. Benzocaine maintains a fair margin of safety, although this diminishes at higher temperatures. Its efficacy remains unaltered by water hardness or pH variations. Similar to MS-222, benzocaine exhibits fat solubility, potentially prolonging recovery times in older fish or gravid females. Dosing between 100-150 mg/L induces comprehensive anaesthesia, with prompt recovery within the desired timeframe.

The advantages of benzocaine (ethyl para-aminobenzoate) include its effectiveness as a fish anaesthetic, rapid induction and recovery times, and a commendable safety margin, particularly for salmonids. Other merits encompass its low cost, widespread availability, ease of handling, and safety.

The disadvantages are Benzocaine is not approved by the FDA for use on food fish in the U.S. as it causes methemoglobinemia in several species of animals so no longer in clinical practice.

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However, it has several disadvantageous features including high price and possible danger to the health of operatives.

Quinaldine

Quinaldine, a yellowish, oily liquid characterized by limited water solubility, necessitates pre-dissolving in acetone or alcohol before being combined with water. In contrast, Quinaldine sulfonate, appearing as a pale yellow, water-soluble powder; represents a more expensive alternative to quinaldine or MS-222.

The acidic nature of quinaldine solutions is typically mitigated by buffering with sodium bicarbonate. Induction is a process lasting 1 to 4 minutes and may induce mild muscle contractions. The effective treatment concentration of quinaldine solutions varies across species, generally ranging from exhibiting anaesthesia at concentrations of 10-50 mg/L, while non-elasmobranch species require 50-100mg/L for the same effect. Grass carp (*Ctenopharyngodon idella*) lose equilibrium within 5 minutes of exposure to 15 mg/L of quinaldine. Notably, higher concentrations, ranging from 50 to 1,000 mg/L, are necessary to achieve complete anaesthesia in Tilapia.

While quinaldine may fall short in inducing the profound anaesthesia required for surgical procedures due to the retention of some reflex responsiveness, higher doses, reaching up to 150 mg/L, have been employed for such purposes, although it is generally not recommended. Unlike MS-222, quinaldine does not typically cause a cessation of gill ventilation in fully anaesthetized fish, reducing susceptibility to asphyxia from respiratory arrest. Notably, the potency of quinaldine is heightened in hard and warm water conditions.

One distinct advantage of quinaldine lies in its rapid recovery profile, making it a cost-effective and favoured tool in activities such as collecting tropical fish for the aquarium trade, as well as in the bait and sport fish industries. However, it is essential to acknowledge certain disadvantages, including its potential to irritate fish, an unpleasant odour, and its classification as a carcinogen.

2-Phenoxyethanol

2-Phenoxyethanol, characterized by its opaque, oily liquid form, demonstrates moderate solubility in water but is freely soluble in ethanol. This property renders it bactericidal and fungicidal, making it a valuable asset in surgical applications. With a relatively broad safety margin, 2-Phenoxyethanol exhibits a spectrum of effects, ranging from light sedation to surgical anaesthesia, typically achieved at concentrations of 100 to 600 mg/L.

The advantages of 2-phenoxyethanol include its cost-effectiveness, sustained activity in diluted solutions for a minimum of 3 days, and its safety for prolonged sedation during activities such as transport.

However, a notable drawback of 2-phenoxyethanol is its relatively low therapeutic index, rendering it toxic and potentially harmful to humans. Handlers may experience fatigue and drowsiness, particularly in poorly ventilated rooms.

Adverse effects associated with its use encompass reduced ventilation, decreased heart rate and blood pressure, reduced blood partial pressure of O_2 coupled with increased CO_2 levels, reduced blood pH, and elevated plasma levels of adrenaline and glucose.

Metomidate

Metomidate, widely employed in human medicine, proves to be an effective anaesthetic for fish, offering the distinct advantage of inducing anaesthesia without the typical stress associated with elevated heart rates. Notably, the induction process is remarkably rapid, occurring within 1 to 2 minutes, and the recovery period is shorter compared to that of MS-222. One of the key advantages of metomidate is its reported potency, particularly in larger fish adapted to seawater, showcasing heightened efficacy in comparison to freshwater fingerlings.

Clove oil

Clove oil, widely recognized for its application in human dentistry and as a food flavouring agent, finds use as an anaesthetic in fish, despite lacking approval for species intended for human consumption or release into public waterways. Its primary constituent, the oil Eugenol, comprising 70 to 90 percent by weight, imparts the characteristic odour and flavour to clove oil. Recovery time is influenced by both higher doses and longer exposure. Comprising approximately 50 percent isoeugenol and 50 percent polysorbate 80, a dosage of 20 mg/L proves effective for most fish species, with induction deemed “stress-free” due to cortisol suppression.

Clove oil offers advantages in terms of cost-effectiveness and ease of use. Notably, it boasts a high margin of safety. However, it necessitates a comparatively extended recovery period when compared to MS-222. A drawback lies in its potential negative impact on growth with repeated use, indicating that the physiology of fish may be influenced by alterations in haematology and biochemistry following exposure.

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Carbon dioxide

Carbon dioxide CO_2 has served as a longstanding and effective anaesthetic, particularly in the context of fish transport. Due to its high solubility in water, CO_2 can be easily diffused into the water as a gas. The efficacy of carbon dioxide anaesthesia has been demonstrated in rainbow trout, with concentrations of 120 to 150 mg/L for fingerlings and 200 to 250 mg/L for adults proving effective. In a study by Peak (1998), the effectiveness of sodium bicarbonate and acetic acid in inducing anaesthesia was compared in smallmouth bass (*Micropterus dolomieu*), northern pike (*Esox lucius*), and lake stugeon (*Acipencer fulvescens*). Additionally, Durborow and Mayer (unpublished data) found that largemouth bass (*Micropterus salmoides*) reached stage 2 anaesthesia within 6 minutes when exposed to a 0.67-g/L NaHCO3 solution (30 L water, 20 g NaHCO3, and 7.5 mL acetic acid) at 6 °C, and recovering in 10 to 15 minutes after 1-hour anaesthesia. Buffering the water with sodium bicarbonate helps reduce hyperactivity and stress. While sodium bicarbonate (NaHCO3) and acetic acid can be used to generate CO_2, the challenges of adjusting and maintaining the required pH make alternative methods more appealing for many procedures.

A notable drawback of carbon dioxide as an anaesthetic is its requirement for a relatively prolonged induction time, typically around 5 minutes at concentrations ranging from 120 to 640 mg/L. This extended induction period may pose practical challenges, especially in situations where swift and precise anaesthesia is essential. Furthermore, controlling the final concentration of CO2 can prove somewhat challenging, introducing an element of unpredictability to the anaesthesia process.

Anaesthetic and Pre-anaesthetic Considerations

- Baseline behavioural parameters, including ventilation, caudal fin stroke rate, and overall activity level, must be meticulously recorded. Prior to anaesthesia, it is imperative to cease feeding the fish. A fish with a full stomach may regurgitate during anaesthesia, potentially leading to the partial clogging of its gills and water contamination.

- Prior to commencing any procedure, it is essential to ensure the availability of distinct water supplies for anaesthesia induction, anaesthesia maintenance, and anaesthesia-free water for recovery. The physical parameters, such as temperature, and chemical variables, including pH, and salinity, in the water used must align with those of the fish's source

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water. Additionally, it is crucial to maintain dissolved oxygen levels above 5 ppm (mg/l), with the optimal range being 6-10 ppm.

- To ensure a smooth anaesthesia event, it is imperative to minimize stress on the fish, as an excited fish may not respond well to anaesthesia. The use of dim lights can effectively reduce stimulation and contribute to a more controlled environment for the fish.

- Handle the fish with utmost care, minimizing any unnecessary contact to prevent abrasions and loss of protective mucus.

- The anaesthesia water needs to be well aerated because, under anaesthesia, breathing (gilling) will be reduced.

- When inducing fish with anaesthesia, be mindful of a potential excitement phase as inhibitory neurons are depressed before achieving anaesthesia; hence, it is advisable to use a cover on the induction tank. Ensure the continuous moisture of the fish's skin and fins during any out-of-water experience. Develop a comprehensive plan to prevent the drying of the skin, fins, and eyes during out-of-water procedures, incorporating measures such as clear plastic drapes and regular tissue rinsing with water from a bulb syringe or a small portable atomizer.

- Personal protective gear must be available. The use of a respiratory mask may be appropriate when measuring powdered anaesthetics, and the use of gloves limits systemic absorption and reduces the potential for zoonotic disease transmission.

Route of administration of anaesthesia

The administration of anaesthetic agents to fish is a nuanced process, contingent upon the specific drug and prevailing circumstances. Depending on the nature procedures, anaesthesia is generally achieved by immersing the fish in an MS-222 bath. For longer procedures, a continuous delivery can be employed by pumping anaesthetic-containing water over the gill.

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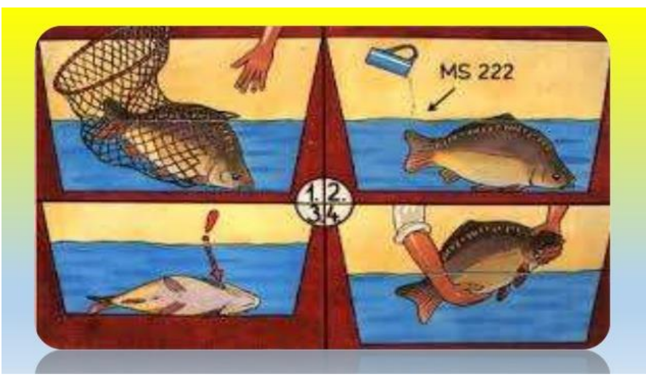
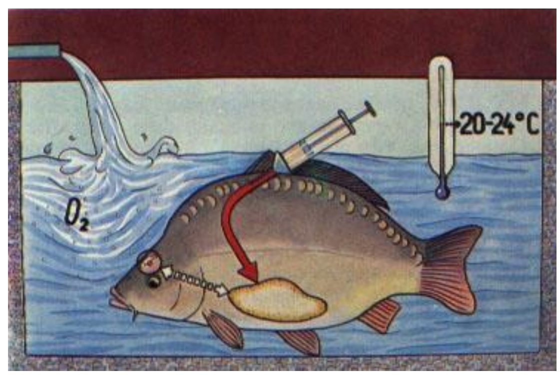
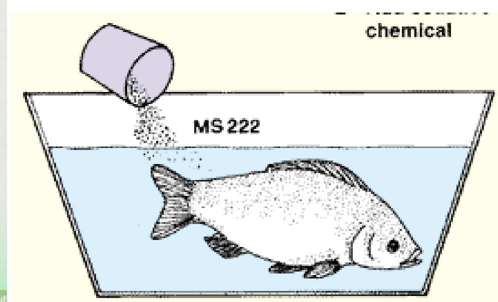
Fish are typically anaesthetized by immersing them in an anaesthetic bath containing a suitable concentration of the drug, facilitating absorption through the gills and rapid entry into the bloodstream. A straightforward method involves preparing the required drug concentration in an aerated container and quickly but gently transferring the fish to the container. Both the anaesthetic bath and the recovery tank should use water with a similar temperature and chemistry to that from the environment in which the animals originated. The main considerations revolve around maintaining optimal temperature, adequate dissolved oxygen, minimal ammonia levels, and reducing faecal matter. In situations where immersion is impractical, especially with larger animals, applying an anaesthetic solution to the gills using a spray bottle can be advantageous. For instance, a solution of MS-222 at a concentration of 100 to 200 mg/L has been reported as effective when applied to the gills of salmonid broodstock. This method enables fish handling without immersion and does not adversely subsequent egg-hatching success.







Synergistic effect of anaesthetics

Supra-additivity or synergism occurs when a mixture of two or more drugs produces a greater response than expected. Drug synergism can be expected when drugs that act by different mechanisms of action are mixed together. Various drug combinations (e.g., nonsteroidal anti-

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inflammatory drugs [NSAIDs] and opioids; opioids and α2-agonists; opioids and local anaesthetics; opioids and dissociative anaesthetics) frequently demonstrate synergistic effects. Synergistic drug combinations are the basis for many multimodal drug combinations but must be administered carefully because unwanted and potentially toxic effects may also be potentiated (e.g., respiratory depression).

Anaesthesia of aquatic invertebrates

Limited information is available regarding the anaesthesia of invertebrates due to less frequent practice. In crustacean culture, culture many operations can be carried out without anaesthesia, although many operations can be carried out with the rapid movement of shrimp can during handling and their cannibalistic tendencies, particularly during holding and transportation. As a result, there has been a growing interest in researching crustacean anaesthetics, primarily in the context of transportation. Crustaceans exhibit distinct responses to anaesthesia compared to finfish, potentially due to differences in synaptic receptor sites that are not affected by certain anaesthetics. For example, MS-222 proves ineffective on numerous crustaceans, often necessitating significantly higher concentrations for anaesthesia compared to fish. Carbon dioxide emerges as an effective anaesthetic for most crustaceans, commonly administered as a mixture of baking soda and acetic acid. Additionally, cooling represents an efficient method to immobilize crustaceans, as excessive cooling can have lethal effects on the animals.

Stages of anaesthesia

Stage 1-Slight loss of reactivity

Stage 2-Reduced respiratory activity

Stage 3-Deep sedation, loss of equilibrium but still responding to stimuli

Stage 4- No response to stimuli but mild respiration

Stage 5- Irregular respiration does not respond to stimuli

Induction

Many anaesthetics can induce various levels or stages of anaesthesia, encompassing sedation, anaesthesia, surgical anaesthesia and ultimately, death. The specific stage attained typically hinges on the administered dose and the duration of exposure. During the initial administration or induction of an anaesthetic, fish may briefly exhibit hyperactive for a few seconds.

Maintenance

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After achieving the desired level of anaesthesia, there may be a need to sustain fish in that state for a specific duration. Maintaining a consistent depth of anaesthesia proves challenging due to the cumulative nature of drug dose and exposure time. This challenge arises from the continued accumulation of anaesthetic levels in the brain and muscles even after blood levels have reached equilibrium. To sustain the desired level of anaesthesia, adjustments can be made by reducing the dosage.

During this maintenance period, it is essential to visually monitor the condition of the animals. A noticeable change in breathing rate is the most obvious indicator of over-exposure. In the event of such signs, prompt action is necessary, requiring either the relocation of the animals or an immediate flushing of the systems.

Recovery

In the recovery stage, the withdrawal of the anaesthetic leads to the fish returning to a normal state. The transition from the anaesthetic medium to the regular rearing medium, known as the recovery bath, is typically undertaken. To reduce recovery time, it is crucial to ensure swift induction and minimal handling time. The initial recovery may take from a few seconds to several minutes, contingent upon the specific anaesthetic used. During the recovery stage, meticulous care is essential to minimize stress and avert mortality. If an animal exhibits difficulty in recovering, enhancing the flow of anaesthetic-free water over the gills will often accelerate and normalize the heartbeat. Techniques such as gently moving the fish back and forth in the recovery bath or softly passing water over the gills with a hose prove effective in augmenting gill blood flow and expediting the elimination of the drug.

Stage	Condition	Behavior/Response
I	Sedation	Motion & breathing reduced
II	Anaesthesia	Partial loss of equilibrium Reactive to touch stimuli
III	Death	Breathing & heart beat stop Overdose - eventual death

Factors affecting anaesthesia

Many factors affect the efficacy of anaesthetics in fish. These can be divided into biological and environmental factors.

Environmental factors

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Temperature: In addition to its effects on metabolism, temperature also affects dissolved oxygen (DO) concentrations. At higher temperatures, there is a reduction in DO levels in the water (Harms 2003), a phenomenon that can intensify anaesthetic-induced hypoxia

pH: The efficacy of immersion anaesthetic solutions is influenced by the pH, wherein a decrease in the pH typically results in reduced efficacy due to increased ionization that interferes with absorption. Immersion anaesthetics with acidic solutions necessitate buffering agents to neutralize the pH, enhancing efficacy while preventing metabolic acidemia. This condition can arise from anaesthetic-induced hypoxemia and anaerobic metabolism. It's worth noting that saltwater, which possesses a higher pH and greater natural buffering capacity compared to freshwater, the addition of buffering agents may depend on the dose of immersion agent, size and activity of the fish undergoing anaesthesia in the same container.

Nitrogenous Compounds: Nitrogenous compounds, such as ammonia and nitrite, have the potential to damage or alter gill morphology, and these changes can have several impacts. They may influence the uptake and clearance of inhalant anaesthetics, compromise oxygen uptake, consequently affecting the metabolism of the anaesthetic agents, and lead to acidemia. Additionally, a significant increase in nitrite may result in methemoglobinemia, thereby diminishing the oxygen-carrying capacity of the blood.

Other factors

Often, the rate at which anaesthetic drugs become effective is related to the gill area to body weight ratio, a parameter that exhibits considerable variation among fish species. Additionally, aquatic species also have diverse metabolic rates, influencing the absorption of chemicals and the induction of anaesthesia. For example, cold-water species appear to respond to lower concentrations of anaesthetic compared to warm-water species

Some factors can influence anaesthesia within a particular species. Typically larger individuals require a higher concentration of anaesthetic compared to smaller ones. In contrast, it has also been reported that the larger, more active fish in a group are anaesthetized faster than smaller ones.

Numerous drugs such as MS-222 and benzocaine, exhibit fat solubility. Consequently, in larger fish or gravid females, the duration of anaesthesia may be prolonged, and recovery may be proceeded at a slower pace as the drug is gradually released from the lipid reserves. Moreover, it

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is noteworthy that diseased or weakened animals are much more susceptible to anaesthetic treatment.

Environmental factors significantly impact the effectiveness of certain anaesthetics. Aquatic invertebrates and fish, being ectothermic, have body temperatures linked closely to their environment. Consequently, the physicochemical passage of the drug into the fish is temperature-dependent. For substances like MS-222, benzocaine, and 2-phenoxyethanol, higher doses or prolonged exposure times are often necessary at lower water temperatures due to decreased absorption rates. Additionally, the pH of an anaesthetic solution can influence efficacy, particularly evident with quinaldine, which loses effectiveness in solutions with low pH.

Legal aspects

Numerous chemical anaesthetics have been employed in fish, but many are now obsolete or infrequently used. The U.S. Food and Drug Administration (FDA) regulates the use of chemicals on food fish. Anaesthetics leave residues in fish flesh, requiring a specific withdrawal time before consumption or release into the environment. Anaesthetics for food animals undergo a rigorous FDA drug development program to safeguard cultured animals, human users, the food chain, and the environment. This process involves collaboration among drug companies, researchers, national agencies, and the farming and feed industries. Licensing new drugs is time-consuming and costly, and the aquaculture industry's smaller scale poses challenges for drug companies. Currently, the only FDA-approved anaesthetic for food fish is tricaine methanesulfonate (MS-222).

Environmental and Other Factors

Drug Concentration/Dosage The administration of immersion agents in aquatic environments presents a nuanced relationship between drug concentration, induction, and recovery times. Typically, a higher, drug concentration or dosage tends to decrease induction and prolong recovery periods. However, this trend is not universal, as exceptions exist. Careful dosing is essential due to the persistence of certain immersion drugs in the brain and muscles beyond blood equilibration. This unique characteristic may lead to a gradual deepening of anaesthesia and, in some cases, respiratory arrest during the recovery phase in anaesthetic-free water.

Novel anaesthetic drug delivery system

Novel drug delivery systems have been developed to prolong the duration of local anesthetic effects and enhance their efficacy. Liposomal formulations, biodegradable polymers,

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and hydrogels have been utilized to encapsulate local anesthetics, providing sustained release and prolonging the duration of pain relief.

Ethical consideration

Ethical issues on animal experimentation started in 1959, where the emphasis has been given on principles of 3Rs, reduction, refinement, and replacement of animal use. According to this principle, minimum necessary numbers of animals are to be used for scientific experiments i.e. reduction. Pain or distress of the animals during experiments has to be minimized, i.e. refinement. Wherever applicable replacements of the animals are to be done with other non-animal alternatives, i.e. replacement.

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