Humane Euthanasia Techniques for Ornamental Fish

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Executive Summary

The Australian Animal Welfare Strategy (AAWS) is a Federal Government initiative under the responsibility of the Department of Agriculture (DA). AAWS was developed to provide both national and international communities with an appreciation of animal welfare arrangements in Australia.

The Aquarium Vet, under the direction of the Pet Industry Association of Australia (PIAA), has been contracted by the DAFF to undertake a review of Humane Euthanasia Techniques for Ornamental Fish. Ornamental fish are defined as fish that are kept at home either as aquarium or pond fish. The vast majority of these fish are less than 30 centimetres (cm) in size, and in fact most are under 15 cm in size.

Currently there is a lack of readily available information in Australia on humane euthanasia techniques for 'sick' ornamental fish for their owners. The aim was to identify a variety of techniques and practical methods to euthanize ornamental fish that are, or could be, made available to the owners of such fish and that improve animal welfare for the fish being euthanized. In the context of this document, owners are either, (1) people working in retail pet and/or aquarium shops, or (2) the actual pet fish owner. A comprehensive literature review was undertaken, as well as contacting international ornamental fish industry organisations to determine the science and techniques available for humane euthanasia of ornamental fish.

The literature search revealed very little data specific to the euthanasia of ornamental fish species with one exception. That exception is the zebrafish (*Danio rerio*) which is a small tropical aquarium fish that in the past twenty years has become a major medical and biological research animal.

As there was little specific information found in the literature, other methods were considered. In evaluating these other various methods of euthanasia several criteria were used, including that the method needed to cause a rapid loss of consciousness - followed by death, the method was reliable and irreversible and that any drugs or compounds recommended were readily available and did not require a veterinary prescription.

In Australia, all of the animal welfare and veterinary surgeon related legislation is state and territory based rather than federal based. A review of the animal welfare legislation showed no reason why owners are not permitted to euthanize their fish if they are suffering, as long as it done in a humane manner. Contact was made with all veterinary surgeon boards. For all states and territories (except Queensland and Australian Capital Territory), what was proposed did not contravene any veterinary surgeon legislation, the key points being that the person carrying out the euthanasia did not charge a fee and that a non-prescription medication

was used. In Queensland for an owner of a fish, to euthanize their fish may actually contravene the *Veterinary Surgeons Act 1936* and the *Veterinary Surgeons Regulation 2002*. This legislation is currently under review and it is hoped that this will be altered to allow owners to euthanize their own fish as in other states and territories. The Australian Capital Territory Veterinary Surgeons Board did not respond to multiple enquiries.

Fish are an incredibly diverse group of organisms and for the purposes of this review were divided into four broad physiological groups - freshwater tropical, freshwater temperate, marine tropical and marine temperate. There are also different sizes which may affect the manner of euthanasia – these can be grouped into small (less than 5cm in length), medium (5 – 15cm) and large (greater than 15cm).

An overdose of anaesthetic in the water is the method most commonly recommended by veterinary and scientific authorities in Australia and overseas for the euthanasia of fish in research environments or by registered veterinarians. Whilst clove oil appears to be an effective euthanasia agent for ornamental fish, the fact that there is not a registered product available means that a firm recommendation cannot be made supporting its use. One recommendation is to encourage the development of an Australian Pesticides and Veterinary Medicines Authority (APVMA) registered (non-prescription) product available to fish owners. A clove oil derivative (AQUI-S®) that is currently registered for fish is only available in volumes that are possibly excessive for the use as we are discussing.

Physical methods have the advantage of not requiring a prescription or the purchase of chemical agents, but do require skill and experience to be performed safely and humanely. In the case of pet fish there is also likely to be a reluctance to use aesthetically displeasing methods on an animal to which there is an emotional attachment. Percussive stunning, decapitation, and pithing are all used either alone or in combination to humanely euthanize fish. Most retail outlets and ornamental fish owners do not possess the ability or the dedicated space to conduct these physical methods.

Hypothermal shock (rapid chilling) via the use of ice slurry can be effective, but research has only validated this method as being effective for some specific species of fish. Based on this research, it appears that hypothermal shock is only suitable for small-bodied tropical fish (either freshwater or marine) due to the sudden temperature difference. With temperate (or cold-water fish) that can survive very low temperatures, the temperature difference is not sufficient to ensure a rapid death or in fact death at all. A detailed step-by step process for using rapid chilling / ice slurry for the euthanasia of small-bodied tropical ornamental fish is presented.

Several methods examined were considered unacceptable for euthanasia of ornamental fish - these included the use of carbon dioxide, exsanguination, freezing, flushing and maceration.

Until further research is conducted, the best and preferred method for the euthanasia of ornamental fish in the home and retail environment is the use of a registered clove oil derivative (such as AQUI-S®) which can be added to the water in concentrations sufficient to result in an anaesthetic overdose. Such a compound covers all possible fish types. The availability in small volumes though is an issue that needs to be considered.

1. Introduction

The Australian Animal Welfare Strategy (AAWS or 'the Strategy') is a Federal Government initiative under the responsibility of the Department of Agriculture (DA). AAWS was developed to provide both national and international communities with an appreciation of animal welfare arrangements in Australia. It also outlines the future direction and improvements to animal welfare in Australia. [1]

The Strategy's vision is that: "The welfare of all animals in Australia is promoted and protected by the development and adoption of sound animal welfare standards and practices." Six working groups have been established as part of the Strategy, covering all animal application sectors. One of these, the Aquatic Animal Working Group (AAWG) has the responsibility to develop and implement the action plan for the aquatic animal sector. Initially this has been restricted to finfish. The AAWG has established a set of Overarching Principles against which sub-sectors can build their specific best practice guidelines to achieve animal welfare (see Appendix One).

Defining fish welfare

Animal welfare is not a new concept. The Book of Proverbs (written nearly 3000 years ago) states 'A righteous man cares for the needs of his animal' (Proverbs 12:10).

The following is taken from a prior AAWS document one of the authors and a reviewer of this project were involved with. *A Review of Current Welfare Arrangements for Finfish in Australia* (Panaquatic 2006) states that –

A definition of animal welfare is not easy to produce because the concept is complex and the word 'welfare' is used in a number of different ways (Appleby 2002). The same applies to the definition of 'well-being'. This articles states that 'there is no simple answer to the question "What is well-being?"' Broadly, animal welfare deals with the humane treatment of animals (Håstein et al 2005). Human beings may affect the welfare of fish in many ways, some easily defined as having welfare implications (e.g. how a fish is killed) whereas others are not so easily identified (e.g. whether or not an activity can be described as causing 'stress' to a fish, such that the well-being of the fish is adversely affected).

The principles of animal welfare have emerged primarily in terrestrial animals, many of which have similar anatomies, physiologies and behaviours (which are often also shared by humans). Fish on the other hand are far more diverse particularly with respect to habitat and ecological niches. In contrast to terrestrial animals, fish are poikilothermic meaning that their internal body temperature is not maintained at a constant temperature but fluctuates

[1] See AAWS website at < http://www.daff.gov.au/animal-plant-health/welfare/aaws>

depending on the temperature of the water in which they live. A great deal of what we need to know regarding fish welfare is yet to be discovered as the scientific study of fish welfare is at an early stage compared to research efforts on other vertebrates.

Ornamental Fish

For the purposes of this document, the term ornamental fish is defined as including both freshwater and marine teleosts (bony fish) and elasmobranchs (cartilaginous fish) that are kept at home either as aquarium or pond fish. The vast majority of these fish are teleosts and are less than 30 centimetres (cm) in size, and in fact most are under 15 cm in size. This document does not refer to larger specimens (greater than 30 centimetres) that may be found in public aquaria.

The ornamental fish industry in Australia involves the sale of a large number of fish. The current estimate is that in excess of 30 million 'tails' (the industry term for a fish) are sold annually of which 40% are produced locally and the remainder are imported (Savage 2013). The industry expects the hobby to continue to grow for a number of reasons, including –

- changes in Australia's demographic living arrangements (i.e. an increase in housing density) in which fish are a convenient pet for small houses and apartments
- increased popularity of marine aquariums due mainly to advances in filter technology allowing once 'difficult to keep' species to now be readily housed.

Project Outline

The aim was to identify a variety of techniques and practical methods to euthanize ornamental fish that are, or could be, made available to the owners of such fish and that improve animal welfare for the fish being euthanized. In the context of this document, owners are either, people working in retail pet and/or aquarium shops or the actual pet fish owner (including fish hobbyists). The project has identified gaps in the literature available where further research may be required.

The Aquarium Vet, under the direction of the Pet Industry Association of Australia (PIAA), has been contracted by the DA to undertake this project. A comprehensive literature review has been undertaken, as well as contacting international ornamental fish industry organisations to determine the science and techniques available for humane euthanasia of ornamental fish.

Knowing humane methods to euthanize ornamental fish is important for the owners of these fish, be they working in retail outlets or members of the general public. Currently there is a lack of readily available information in Australia on humane euthanasia techniques for 'sick' ornamental fish for their owners.

Unlike killing fish in commercial fisheries (wild-capture and aquaculture industries), which are generally species specific and target a certain life stage, ornamental fish cover a wide range of species sizes and ages and it was expected that multiple methods of euthanasia may be required to address this range of fish. In addition, commercial fisheries generally focus on techniques used for killing large numbers of fish at a time during harvest (slaughter) activities, whereas the ornamental industry generally requires techniques for owners to kill individual fish.

There is anecdotal information suggesting that some of the methods currently used for ornamental fish are inhumane. This project has considered the techniques and methods that are currently used, summarised the science as it is known and proposed practical techniques that are humane and promote welfare awareness within the ornamental fish industry.

The project acknowledges one of the overarching principles established and endorsed by the AAWS AAWG (see Appendix One - number 4) i.e. For fish held in captivity, any visibly damaged or sick fish should be assessed and either treated appropriately or promptly removed for killing by humane means suitable for the species.

Researching information regarding correct euthanasia techniques for ornamental fish species and making this information publically available and well communicated will help people make ethical decisions regarding animal welfare, supported by knowledge and skills. The project will help ensure that the welfare needs of ornamental fish are understood and met. Liaisons with other international peak bodies for ornamental fish (e.g. Ornamental Aquatic Trade Organisation and Ornamental Fish International) will encourage partnerships and contributions to animal welfare globally.

In summary this project makes recommendations for the humane euthanasia of sick fish either in a retail environment or for the home owner that are practical and protect the welfare of the fish involved.

2. Humane Euthanasia

The term euthanasia comes from the Greek language meaning a good death (*eu* = good, *thanatos* = death). In a recent document, *AVMA Guidelines for the Euthanasia of Animals*, the American Veterinary Medical Association (AVMA) states that the term is usually used to describe ending the life of an individual animal in a way that minimizes or eliminates pain and distress (Leary et al. 2013). Euthanasia of animals as a means to end suffering and pain is widely accepted as good animal welfare.

The Oxford Dictionary defines the term humane as **having or showing compassion or benevolence** such as *regulations ensuring the humane treatment of animals* and inflicting the minimum of pain such as *humane methods of killing* (Oxford Dictionary 2013).

The humaneness of the actual technique employed is an important ethical issue. We must ensure that if an animal's life is to be taken, it is done with the highest degree of respect, and that we aim to make the death as distress free as possible (Leary et al. 2013). This document also states that the euthanasia process should minimize or eliminate pain, anxiety, and distress prior to loss of consciousness.

In evaluating the various methods of euthanasia the following criteria were considered –

a) **The method needed to cause a rapid loss of consciousness, followed by death**

A loss of consciousness refers to a loss of awareness of and responding to one's environment. Whichever technique is used, a rapid loss of consciousness is essential. This should be quickly followed by cardio-respiratory arrest and finally a loss of brain function must be achieved. At all times the correct and gentle handling of the fish to reduce stress is essential.

b) The method was reliable and irreversible

The method had to be reliable and irreversible so that it was not possible for a fish to later recover and then be out of water and extremely distressed, and potentially die in an inhumane manner.

c) Any drugs or compounds recommended were readily available and did not require a veterinary prescription

One of the common concerns that many ornamental fish owners have with regards to the euthanasia of a sick fish is the cost of a veterinary visit in comparison to the actual or perceived value of the fish involved. Whilst there are some fish owners that do take their fish to veterinarians for examinations and euthanasia, this is still uncommon. Therefore, it is

important that any drugs or medications that are recommended be readily available and do not require a veterinary visit or a prescription.

d) There was no risk to the people involved in the process

Any recommended technique must be safe to use by non-veterinary people so that there is no risk of any harm to the person performing the euthanasia. See Section 4.4 for Occupational Health and Safety comments.

e) The method was acceptable to members of the general public

Any recommended technique must be acceptable to a person who has no prior experience. The technique should not be overly challenging to implement.

The main reason for considering the euthanasia of a fish will be that it appears to be suffering or terminally ill by the owner. In some situations, it may be that the fish can no longer be kept and cannot be re-homed. Whilst most owners are not experts in the diagnosis of a sick or dying fish, there is anecdotal evidence suggesting that when an owner thought that a fish was sick or dying, the method of disposal was by flushing it down the toilet or using some other crude methods. As discussed below (Section 4.3) many of these are not acceptable methods.

It is recommended that if the owner of the fish is inexperienced, before deciding to euthanize the fish, they seek expert advice from a reliable pet or aquarium retail outlet or their local veterinarian.

Confirming Death

The confirmation of death in fish is not an easy process. A lack of respiration (opercular movement) for at least 30 minutes would be consistent with death. Very few other signs are present to confirm death in the situation we are discussing, where there is a lack of access to equipment such as an ultrasound or Doppler to detect a heartbeat. Certainly a lack of a corneal reaction (tapping the surface of the eye) or pinching the tail fin will indicate deep anaesthesia but not confirm death. These tests are discussed in more detail when examining the stages of anaesthesia under Section 4.2.1 Chemical Methods. Establishing death is discussed in more detail as it applies to the various methods of euthanasia which are discussed in Section 4.2.

Disposal of the Body

Whilst humane euthanasia is the topic for this document, it is important to mention disposal of the fish body afterwards. Correct disposal is important to minimize any possible risk of disease spreading to fish populations in local rivers and streams.

There are two alternative methods. The first is to wrap the body in a paper bag and bury it in the garden. This would need to be deep enough that foraging birds or animals could not find and eat the body, with greater than 30 centimetres being sufficient. It is important that the burial site is not located near waterways. The second is to wrap the body in a plastic bag and place this into rubbish that will end up in a landfill situation, such as normal commercial or residential rubbish collection. If it is going to be some time after the death to the rubbish collection, it will be advisable to freeze the body until the time of the rubbish collection.

It is not acceptable to flush bodies down the toilet. Whilst the risk of disease spread is negligible due to the effects of dilution, it is not necessary when there are other more suitable methods of disposal. The same applies to the disposal of the body in natural waterways or simply throwing the body in the garden.

3. Legislation

With regards to making recommendations, it is essential to not contradict any animal welfare and veterinary surgeon related legislation.

In Australia, all of the appropriate legislation is state based rather than federal based. This means that there are six states and two territories to examine.

Welfare Legislation

Each state and territory has its own legislation that relates to animal welfare. Traditionally these were Prevention of Cruelty to Animals Acts and are often called POCTAs. However, they now have many different names and are listed in Table 1 below.

A review of this legislation shows no reason why owners are not permitted to euthanize their fish if they are suffering, as long as it done in a humane manner.

Table 1 – State and territory POCTA legislation

State / Territory	**Appropriate Legislation**
New South Wales	*Prevention of Cruelty to Animals Act 1979 No. 200*
Queensland	*The Animal Care and Protection Act 2001*
South Australia	*Animal Welfare Act 1985*
Tasmania	*Animal Welfare Act 1993*
Victoria	*Prevention of Cruelty to Animals Act 1986*
Western Australia	*Animal Welfare Act 2002*
Australian Capital Territory	*Animal Welfare Act 1992*
Northern Territory	*Animal Welfare Act*

Veterinary Surgeon Legislation

Contact was made with the governing body (boards) of veterinary surgeons in all states and territories (see Table 2) to seek clarification regarding the humane euthanasia of ornamental fish. The following information was provided -

The project aims to identify practical euthanasia methods that improve animal welfare for ornamental fish euthanized by their owners –

- *owners of retail aquarium / pet shops*
- *pet fish owners*

As a part of this review I wish to seek clarification that the act of euthanasia of a fish is not considered an act of Veterinary Science and as such can be carried out by non-veterinarians (providing of course that a fee is not charged for the service of the euthanasia) and that the use of a medication (non-prescription) such as clove oil or a derivative is not contradictory to any Veterinary Surgeons Act.

Table 2 – State and territory veterinary surgeon boards and legislation

State / Territory	Governing Body	Legislation
New South Wales	Veterinary Practitioners Board of New South Wales	*Veterinary Practice Act 2003*
Queensland	Veterinary Surgeons Board of Queensland	*Veterinary Surgeons Act 1936* *Veterinary Surgeons Regulation 2002*
South Australia	Veterinary Surgeons Board of South Australia	*Veterinary Practice Act 2003*
Tasmania	Veterinary Board of Tasmania	*Veterinary Surgeons Act 1987*
Victoria	Veterinary Practitioners Registration Board of Victoria	*Veterinary Practice Act 1997*
Western Australia	Veterinary Surgeons Board of Western Australia	*Veterinary Surgeons Act 1960*
Australian Capital Territory	Veterinary Surgeons Board ACT	*Health Professionals Act 2004*
Northern Territory	Veterinary Board of the Northern Territory	*Veterinarians Act*

All boards replied (with the exception of the Australian Capital Territory – see below) and have indicated that what is proposed would not contravene any veterinary surgeon legislation, with the exception of Queensland where the advice was –

The Board advises that any treatment that changes the physiological condition of an animal (including euthanasia) can be considered an act of veterinary science unless there is an exemption under the legislation. There can be serious animal welfare issues for persons who euthanase animals.

Examining the appropriate legislation reveals that the euthanasia of fish is not exempt under the legislation and thus for an owner of a fish in Queensland, to euthanize a fish may actually contravene the *Veterinary Surgeons Act 1936* and the *Veterinary Surgeons Regulation 2002*. This legislation is currently under review and it is hoped that this anomaly will be corrected.

Many attempts were made via email and telephone to contact the Veterinary Surgeons Board of the Australian Capital Territory with no success.

Chemical Control

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is a federal government statutory authority established in 1993 to centralise the registration of all agricultural and veterinary chemical products into the Australian marketplace. Previously each State and Territory government had its own system of registration. It is essential that any chemical recommended by this report is approved by the APVMA and as such is unlikely to contravene any State or territory legislation.

4. Methods of Euthanasia

Euthanasia techniques that are considered humane for use in ornamental fish are examined and discussed. In addition, euthanasia techniques that are considered to be unacceptable have been included as a separate section.

Because there are various groups of fish, a technique that is acceptable in one group may not be acceptable in another group. Before we examine the various techniques we need to understand these basic groups of fish.

4.1 Groups of Fish

Fish are an incredibly diverse group of organisms with about 30,000 species recognized (FishBase 2013) that inhabit a huge array of biotopes. Of these, several thousand species are regularly traded in the ornamental industry.

Ornamental fish can be divided into four broad physiological groups depending on their natural water environment conditions. The first variable is water salinity and the second is the water temperature. Regarding salinity there are freshwater (FW) fish such as are found in lakes and rivers and sea water (SW) or marine fish. Salinity is measured in parts per thousand (ppt) of salt. Freshwater is effectively 0 ppt, while sea water is 35 ppt. The internal salt level of fish is approximately 9 ppt. These two groups of fish have different osmoregulatory mechanisms to cope with these two extremes of salinities. Placing a marine fish in freshwater is very stressful due to the osmotic pressure that it is then placed under. Equally if a freshwater fish is placed in sea water this is very stressful for the fish.

There is another small group of brackish water fish that exist in salinities of between 5 and 30 ppt salinity. They are not discussed any further as they are not commonly kept, however it is essential that whichever technique is used they are maintained in the salinity that they are used to.

The second variable refers to the normal temperature of their environment. Even though fish are poikilothermic, each species still has a preferred optimal temperature range. Generally there are two broad groups of fish with regards to their optimum temperature –

- tropical (Tr) fish with a water temperature range of 22 – 30^{o}C
- temperate (Te) fish with a water temperature range of less than 22^{o}C and possibly even down to a few degrees as would occur in outdoor ponds in winter

Combining these two variables we end up with the four broad physiological groups of fish which are listed below with their abbreviations in parentheses –

- Freshwater Tropical (FW Tr)
- Freshwater Temperate (FW Te)
- Marine Tropical (SW Tr)
- Marine Temperate (SW Te)

Because of the physiological differences between these four groups of fish, a euthanasia technique that is humane and effective in one group may not be appropriate in another group of fish, due to osmoregulatory and temperature issues.

The size of the fish may also have an impact as to whether a particular technique of euthanasia is appropriate or not. For simplicity there are three sizes of fish within each of the above four groups –

- Small (less than 5cm) S
- Medium (5 – 15cm) M
- Large (greater than 15cm) L

With the various euthanasia techniques, whilst the salinity of the water is critical to avoiding an osmotic stress to the fish, this is easily prevented by placing the fish in water that is matched for salinity (a sample of the fishes tank water is ideal). Removing this factor and combining the remaining two (water temperature and size) will give six possible combinations as listed in Table 3 with their abbreviations in parentheses -

Table 3 - Abbreviations used to indicate different groups of fish

Water Type	**Size**	**Abbreviation**
Tropical	Small (< 5cm)	Tr S
	Medium (5 – 15cm)	Tr M
	Large (> 15cm)	Tr L
Temperate	Small (< 5cm)	Te S
	Medium (5 – 15cm)	Te M
	Large (> 15cm)	Te L

Because of the substantial differences between these six groups, a euthanasia technique that is humane and effective in one group may not be appropriate in another group of fish. As the various techniques are discussed in the following sections, the abbreviations outlined in Table 3 will be used to indicate that the technique is appropriate.

4.2 Methods of Euthanasia Available

The literature search revealed very little data on the euthanasia of specific ornamental fish species with one exception. That exception is the zebrafish (*Danio rerio*) which is a small tropical aquarium fish that in the past twenty years has become a major medical and biological research animal. They are housed in specific zebrafish "houses" (systems designed for optimal holding conditions) and several papers have discussed humane euthanasia techniques of this species (e.g. Matthews and Varga 2012 and Wilson et al. 2009).

Figure 1 – Zebrafish (D*anio rerio*)

With the exception of the zebrafish, much of the peer-reviewed information available on euthanasia is for large-bodied aquaculture species with limited application to ornamental species. Ornamental species are typically small-bodied and in most cases euthanasia will only be required for one fish at a time.

Four documents that provide general information on euthanasia applicable to ornamental species are –

- New South Wales Fisheries "Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research" (Barker et al. 2002)
- Australia and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART) document "Euthanasia of animals used for scientific purposes" (Reilly, 2001)
- American Veterinary Medical Association (AVMA) "Guidelines for the Euthanasia of Animals: 2013 edition" (Leary et al., 2013)
- Directorate-General of the Environment, Nuclear Safety, and Protection (DGXI) of the European Commission "Recommendations for euthanasia of experimental animals: Parts 1 and 2" (Close et al. 1996, Close et al. 1997).

These documents provide general information on a range of euthanasia methods and their suitability to aquatic species, but are intended as guidance documents for researchers and

veterinarians rather than to provide information for retail pet/aquarium owners and home fish owners.

Euthanasia methods can be separated into two general groups: chemical and physical. Chemical methods, i.e. anaesthetic overdose, are mostly only available to ornamental fish owners when prescribed and administered by a veterinarian. However, there are a few over the counter (non-prescription) chemicals that can be used.

Physical methods have the advantage of not requiring a prescription or the purchase of chemical agents, but do require skill and experience to be performed safely and humanely. In the case of pet fish there is also likely to be a reluctance to use aesthetically displeasing methods on an animal to which there is an emotional attachment.

Chemical and physical methods available for the euthanasia of ornamental fish species are outlined in the following sections.

4.2.1 Chemical Methods

In order to understand the use of chemical options, we need to examine the methods used to induce anaesthesia (placing them to sleep) in fish, of which the most commonly used method is a chemical bath. Another method for applying chemical anaesthesia is the use of injections. These will not be discussed as it is not appropriate in the context of this report. Irrespective of the method of induction of anaesthesia, there are various stages that are well recognised, as the fish falls asleep.

Table 4 – Various stages of anaesthesia in fish

Level Number	**Level of Anaesthesia**	**Signs associated with this Level**
Level 0	Normal	Normal swimming behaviour and reaction to external stimuli
Level 1	Light Sedation	Still swimming but reduced reaction to external stimuli; equilibrium normal; normal opercular rate
Level 2	Deep sedation / Light anaesthesia	No swimming; loss of equilibrium (rolls over = belly up) but may still try to right itself; normal to slightly decreased opercular rate; still maintains a tail reaction*
Level 3	Surgical anaesthesia	Complete loss of equilibrium; complete loss of reactivity (negative tail reaction); very slow opercular rate; slow heart rate
Level 4	Medullary collapse	Total loss of opercular movement followed by cardiac arrest EUTHANASIA STAGE

* Tail reaction involves pinching the tail fin to see if there is a response.

The two factors that affect the depth of anaesthesia are the time and concentration of the agent used. Increasing the concentration of the agent used will affect whether Stage 2 or Stage 4 is reached, as the various stages are a continuum with higher concentrations more likely to achieve Stage 4 than Stage 2. If the fish is to be euthanized, we need to reach Stage 4 as quickly as possible, providing that this does not create stress for the fish (see later - clove oil section).

It is essential when using chemical methods, that at no stage the fish is subject to an osmotic shock. So irrespective of the chemical used, it must be placed into the same water parameters that the fish is residing in. Thus, generally a sample of water from the tank or pond is placed into a smaller container for the euthanasia process. For example for small (S) fish only 2 litres would be required, for medium (M) fish a minimum of 10 litres and for large (L) fish a minimum of 20 litres would be needed.

An overdose of anaesthetic is the method most commonly recommended by veterinary and scientific authorities in Australia and overseas for the euthanasia of fish (e.g. Barker et al. 2002, Close et al. 1997, Leary et al. 2013, Reilly 2001). There are several chemical agents suitable for euthanasia of fish, however in Australia the majority of these agents are only available by veterinary prescription and are therefore not covered in detail in this report. Prescription-only chemical agents considered suitable for euthanasia in fish are summarised in Table 5.

Table 5 – Various prescription chemicals that can be used for euthanasia.

Chemical agent	**Reference**
2-phenoxyethanol	Borski et al. 2003, Close et al. 1997, Leary et al, 2013
Benzocaine	Barker et al. 2002, Borski et al. 2003, Close et al. 1997, DAFF 2009, Leary et al. 2013, Reilly 2001.
Halothane	Close et al. 1997, Reilly 2001,
Isoflurane	Leary et al. 2013
Quinaldine sulfate	Borski et al. 2003, Close et al. 1997, Leary et al. 2013
Sodium pentobarbitol	Leary et al. 2013, Reilly 2001
Tricaine methanosulfonate (MS 222)	Barker et al. 2002, Blessing et al. 2010, Close et al. 1997, Leary et al. 2013, Reilly 2001, Topic Popovic et al, 2012, Wilson et al. 2009.

It is interesting to note that 2-phenoxyethanol is now available commercially in the United Kingdom (UK) and the European Union (EU) under the brand name Aqua-Sed. It is registered for the sedation, anaesthesia and euthanasia of fish and is an open seller (non-prescription). For details of Aqua-Sed see http://www.vetark.co.uk/pages/Euthanasia-of-fish.aspx?pageid=474. One of the references in Table 5 (Close et al. 1997) indicates that 2-phenoxyethanol was inappropriate for euthanasia in fish, stating - *It requires large doses to achieve death with a long induction period. Some species exhibit hyperactivity prior to loss of consciousness*. There is no product registered for fish in Australia that contains 2-phenoxyethanol.

While there are a number of widely acceptable chemical agents available for the euthanasia of fish, there are relatively few non-prescription chemical agents considered suitable for euthanasia of fish that are accessible to ornamental fish owners. However there are a couple of chemicals that are potentially suitable.

Clove oil

Clove oil is an essential oil derived from the clove plant, *Syzygium aromaticum*. It is a clear oily liquid with an extremely aromatic odour and flavour. The active ingredient is eugenol and it is present in clove oil at between 70 to 90% by volume. Clove oil has been used as a mild topical anaesthetic for centuries and in particular is used to ease toothache in people.

Clove oil and its derivatives that utilise iso-eugenol (the active constituent of clove oil) are the most readily available chemical agents suitable for the euthanasia of fish. Clove oil and its derivatives are recommended for the euthanasia of finfish by the AVMA (Leary et al. 2013) and ANZCCART (Reilly 2001) guidelines. A study by Holloway et al. (2004) showed that euthanizing fish with clove oil caused no increase in either cortisol or glucose levels, which are considered the hallmark indicators of stress. Similarly, a recent study by Rahmanifarah et al. (2011) found that clove oil induced rapid anaesthesia and death in common carp (*Cyprinus carpio*), with none of the aversive behaviour observed in response to asphyxia, carbon dioxide (CO_2) or chilling. Clove oil can be purchased over the counter from chemists and health food stores. The purity of clove oil may vary between brands and this can cause some variation in response.

Figure 2 – Bottle of Clove Oil from a chemist

The dose of clove oil recommended for euthanasia is 0.25 - 0.50 ml per 1 litre of water (=250 - 500 ppm). This is higher than the previously reported dose of 150 ppm (Holloway et al. 2004). The rationale for recommending such a high dose is to allow for any potential variation in the purity of the clove oil preparation being used, the relatively poor dissolution of clove oil in water, as well as to ensure that there is no chance of the fish recovering from the euthanasia procedure due to an insufficient dose i.e. the procedure needs to be irreversible.

It is important to add this dose slowly to the water with the fish. If a fish is placed directly into a high concentration of clove oil it appears to distress the fish with rapid swimming etc. (personal observation). It is suggested to add the required dose slowly over a period of five minutes.

Whilst clove oil appears to be an effective euthanasia agent for ornamental fish, the fact that there is not a registered product available means that a firm recommendation cannot be made supporting its use. One proposal is to encourage the development of an APVMA registered (non-prescription) product available to fish owners as is currently available in the UK and EU.

Aqui-S

There is a commercial preparation available (AQUI-S®) that contains purified and standardised concentrations of the active constituent of clove oil (approximately 50% iso-eugenol). This product AQUI-S® (see http://www.aqui-s.com/) may be more effective than clove oil as it is designed to dissolve well in water. It was originally developed for the humane harvesting of salmon in New Zealand.

There is a version that is suitable for the ornamental industry called AQUI-S® 10 Ornamental (see http://www.aqui-s.com/index.php/aqui-s-products/aqui-s-ornamental). It is available in smaller quantities and the website discusses its use for euthanasia. At this stage AQUI-S® 10 Ornamental is not registered or available in Australia.

Because AQUI-S® is a product used predominantly in the salmonid aquaculture industry, it is only available in volumes that are possibly excessive for the use as we are discussing. However, AQUI-S® is a registered and non-prescription product that is available in Australia. It is important that the manufacturer's recommendations are followed for safe handling.

Ethanol

Ethanol has been shown to have a sedative and anaesthetic effect in zebrafish (Gerlai et al. 2000, Peng et al. 2009). The AVMA suggests that ethanol could be used for euthanasia at a dose rate of 10 - 30 mL of >95% ethanol per litre of water, which will cause respiratory collapse with prolonged exposure (Leary et al. 2013). To be effective, ethanol must be at least 95% ethanol per litre (i.e. 95% pure), so fixative grade ethanol (70%) and distilled beverages (~ 40%) are not appropriate for use as an anaesthetic or euthanasia agent in fish. It is unlikely that ornamental fish owners would have access to ethanol of sufficient purity for this method to be appropriate for home use.

Spearmint oil and oil of wintergreen

The combination of spearmint oil and oil of wintergreen has been reported to have anaesthetic properties equivalent to MS-222 in Atlantic salmon (*Salmo salar*) (Danner et al. 2011), but the effectiveness of this product for euthanasia has not been assessed. Although this preparation appears to have potential as a method of humane euthanasia, there is currently insufficient information on the action of this preparation in species other than Atlantic salmon, or on the dosage rates required for euthanasia, to allow recommendation of this method for euthanasia of ornamental fish.

To ascertain that a fish is dead is not easy. It is recommended that the fish be left in the container with the euthanasia solution for a period of at least two hours after it appears that opercular movements have ceased, before removing the body for disposal.

The use of Aqui-S for euthanasia is appropriate for all six groups (see Table 3) providing that the fishes own water (or identical water parameters) is used and a volume of water appropriate to the fish size is used.

4.2.2 Physical Methods

The use of physical methods requires consideration of factors such as environmental conditions and body size, and not all physical methods are appropriate for all species. In addition, physical methods such as decapitation or percussive stunning are aesthetically unpleasant and therefore may not be appropriate for the home owner or even in the retail environment.

Physical methods of euthanasia have been reported to be "acceptable with reservation" (Reilly 2001), “acceptable when the use of anaesthetic overdose is not possible” (Barker et al. 2002) and “acceptable when performed by trained personnel using appropriate equipment” (Leary et al. 2013).

Percussive stunning, decapitation, and pithing

Percussive stunning, decapitation, and pithing are all used either alone or in combination to kill fish for industry or research (Van de Vis et al. 2003). The following describes each one in some detail –

- **Percussive stunning**, also known as cranial concussion or blunt force trauma, involves a rapid blow to the head of the fish with sufficient force to render it immediately insensible or unconscious. The rapid blow could be delivered by a hammer, mallet or a solid piece of wood. This will be very difficult with a small fish.

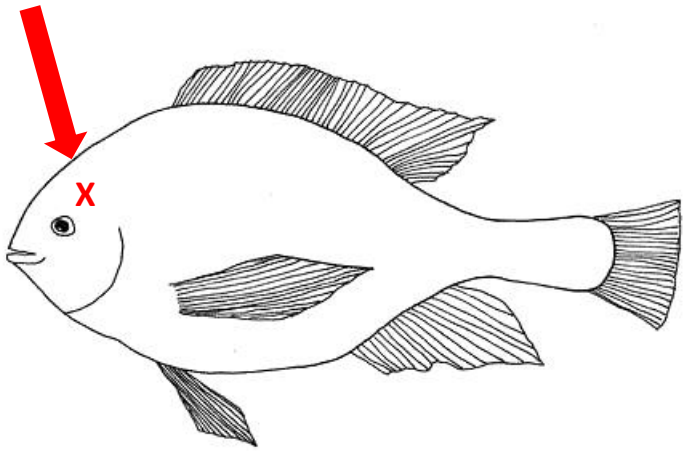

Figure 3 – The site for percussive stunning (arrow and cross marks the actual site)

- **Decapitation** involves separation of the brain from the spinal cord by severing the spinal cord with a sharp knife or scalpel blade to remove or partly remove the head. The back of the head / operculum opening is the site of knife insertion and a rotation

of the wrist once the knife is inserted will assist in the decapitation process. The red line represents the line to cut.

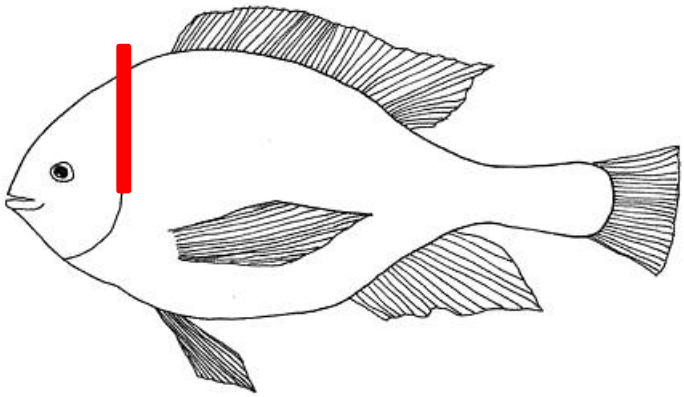

Figure 4 – The site for decapitation (red line)

- **Pithing**, also known as brain spiking or *iki jime*, is a method of killing fish in which a sharp tool (such as a pointy knife or screwdriver) is used to pierce the skull of the fish and destroy the brain. Excellent detail of this process as used in recreational fishing is available at http://www.ikijime.com/. The site of insertion is dorsal (above) and slightly posterior (behind) to the eyes of the fish. There is some variation in the exact site of the brain in fish. However, as the fish we are dealing with are not very large in comparison to the implement being used the exact location is not critical.

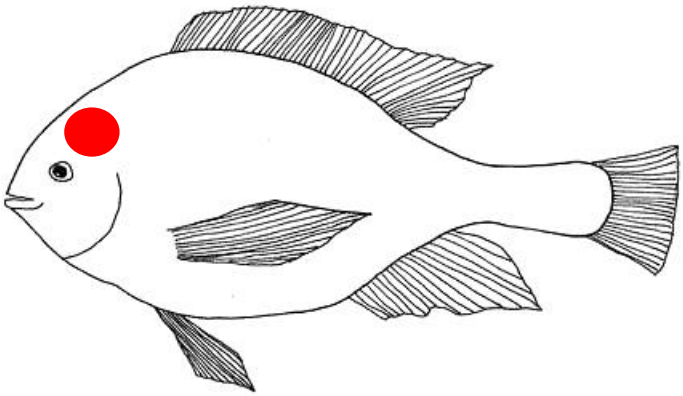

Figure 5 – The site for pithing (red circle)

While a blow of sufficient force can kill the fish immediately (Poli et al. 2005), percussive stunning is generally used prior to other physical methods such as decapitation, exsanguination (bleeding), or pithing to ensure death (this is then called a two-step process). Percussive stunning was found to be more effective than bleeding in ice slurry or electrocution for killing turbot (Morzel et al. 2002). Both the AVMA (Leary et al. 2013) and

ANZCCART (Reilly 2001) state that percussive stunning should be followed immediately by pithing to ensure brain destruction and death, while Close et al. (1997) recommend decapitation to ensure death.

Borski and Hodson (2003) considered decapitation alone to be a suitable method for euthanasia due to a lack of evidence indicating that fish perceive pain or distress following decapitation. Conversely, both the AVMA (Leary et al. 2013) and ANZCCART (Reilly 2001) guidelines require decapitation or cervical dislocation to be performed as part of a two-step procedure for it to be considered acceptable. Leary et al. (2013) states that decapitation is only acceptable if followed by pithing to ensure rapid loss of brain function, while Reilly (2001) considers both methods "acceptable with reservations" and recommends they only be performed on stunned or anaesthetised fish.

The suitability of these methods for euthanasia of ornamental fish species will depend largely on the size of the fish and the willingness and ability of the fish owner to perform the technique efficiently. These techniques are suitable for both tropical and temperate species. Physical methods ideally need to be conducted as a two-step process to be humane and fulfil our criteria as described in Section 2.

Decapitation, followed by pithing, is suitable for Tropical Small, Tropical Medium, Temperate Small and Temperate Medium fish.

Percussive stunning, followed by pithing, is suitable for both Tropical Medium, Tropical Large, Temperate Medium and Temperate Large fish.

Rapid chilling/ ice slurry

Hypothermal shock (rapid chilling) can be used in selected species of fish as a means of humane euthanasia. Studies have found the use of ice slurry to be very effective for small (S = < 5 cm) fish such as zebrafish (*Danio rerio*) (Matthews and Varga 2012), and ice slurry has even been found to be preferable to the anaesthetic agent MS-222 in this species (Wilson et al. 2009). The AVMA (Leary et al. 2013) states that based upon this research rapid chilling is acceptable for euthanasia of small-bodied tropical species such as zebrafish (*Danio rerio*).

However, studies in fish above 5 cm in size (M and L) and species that are cold tolerant indicate that it is not an ideal euthanasia method (Leary et al. 2013). For example, common carp exposed to ice slurry showed aversive behaviour and took up to 48 minutes for opercular movement to stop (Rahmanifarah et al. 2011), and Roth et al. (2009) reported that 1 kg turbot (*Scopthalmus maximus*) that were chilled to -1ºC appeared to be dead but were in fact alive and recovered within 30 minutes of return to holding tanks at 14ºC. Larger-bodied fish (smaller surface area to body size) do not experience a sufficiently rapid hypothermic shock to cause rapid death. Additionally, it has been reported that if the cooling rate is too slow

there is an initial period where ice crystals form on the skin and internally (Wilson et al. 2009) which is likely to cause discomfort in conscious fish.

It is essential that the ice slurry is at a temperature of 2 – 4^{o}C and that the fish comes in contact with this chilled water as quickly as possible. The fish should not come in direct contact with the ice, which may lead to the development of internal ice crystals, and should instead be placed in a depression of the slurry. This will ensure that the entire body of the fish will come in contact with the cold water and not the ice. This method is only suitable for tropical fish (either freshwater or marine) due to the sudden temperature difference. With temperate (or cold-water fish) that can survive very low temperatures, the temperature difference is not sufficient to ensure a rapid death or in fact death at all.

Hypothermal shock has been studied in at least one species of medium (M = 5 – 15 cm) fish – the Australian river gizzard shad or bony bream (*Nematalosa erebi*) (Blessing et al. 2010). However, studies in other fish above 5 cm (M and L) indicate that it is not an ideal euthanasia method (Leary et al. 2013). This is because larger-bodied fish (smaller surface area to body size) do not experience a sufficiently rapid hypothermic shock to cause rapid death. Also, as the time involved before unconsciousness is too long, there is an initial period where ice crystals form on the skin and internally which causes discomfort.

Another potential issue with ice slurry is with marine fish. The melting of freshwater ice into the seawater will drop the salinity and could cause osmotic stress to the fish. However, if the ice slurry is made as detailed below then minimal melting will have occurred before the fish dies.

A detailed step-by step process for using rapid chilling / ice slurry for the euthanasia of ornamental fish can be found in Appendix Two.

Rapid chilling / ice slurry is only suitable for Tropical Small fish.

4.3 Unacceptable Methods

Unacceptable methods of euthanasia include any method -

- that causes unnecessary pain or distress prior to a loss of consciousness
- is humane but aesthetically unsatisfactory
- which presents an unnecessary risk to the person carrying out the procedure

The following methods are considered unacceptable for euthanasia of fish -

Asphyxia in air

Asphyxia in air (i.e. removal of the fish from water) is unacceptable due to the prolonged period of suffering prior to unconsciousness (Close et al. 1997, Leary et al. 2013). It has been reported that some species may take over an hour to become unresponsive when killed using this method (Poli et al. 2005, Bagni et al. 2007, Rahmanifarah et al. 2011).

Carbon Dioxide

Asphyxia with carbon dioxide is unacceptable as it causes distress in fish and can pose an unacceptable risk to the person carrying out the procedure. Effective use of this method requires the ability to regulate the concentration of pure carbon dioxide in the water (Leary et al 2013), which is not possible using the readily available methods often described in the grey literature (e.g. Alker-Seltzer tablets into water).

Although saturation of water with carbon dioxide will result in death much faster than asphyxia in air, it has been reported to cause a strong aversive response in fish prior to death that is likely associated with distress (Close et al. 1997, Van de Vis et al. 2003, Poli et al. 2005, Rahmanifarah et al. 2011).

Cervical Dislocation

This involves breaking the cervical bones in the neck. This method is only suitable for small-bodied fish and is not acceptable in larger fish (Reilly 2001). The authors believe that decapitation is a much preferred technique.

Exsanguination

The process of exsanguination (bleeding to death) on its own as a euthanasia procedure is considered unacceptable. The technique consists of cutting the gills (usually by basically cutting across the throat area) so that the fish bleeds to death. It takes some time for the fish to die and in the meantime it is conscious. As such it does not fit the criteria of a humane euthanasia technique.

Freezing

Death by slow cooling in the freezer is unacceptable as the period prior to death is prolonged (Close et al. 1997, Reilly 2001, Leary et al. 2013). It has also been reported that ice crystals can form in the muscles of fish that are frozen slowly at -20ºC (Wilson et al. 2009), which is likely to cause unnecessary discomfort.

Freezing has been suggested as an adjunctive method for stunned or anaesthetised fish (Leary et al. 2013) but should never be used on conscious fish.

Flushing

Flushing down a toilet is never acceptable for a number of reasons. The exposure of fish to changes in water quality and temperature, along with potential exposure to chemicals, are likely to cause prolonged distress prior to death (Leary et al. 2013). Additionally, the potential release of pathogens from diseased fish into the environment poses an unacceptable risk to wildlife and natural waterways.

Maceration

This method involves placing the fish to be euthanized into a blender or sink garbage disposal unit, and when performed correctly results in almost instantaneous death (Leary et al. 2013). Close et al. (1997) state that fish less than 2 cm in length can be humanely euthanized by maceration with a garbage disposal unit, and Mathews and Varga (2012) reported that maceration with a garbage disposal unit following immobilisation in ice water (2 - 4ºC) was effective for destruction of zebrafish (*Danio rerio*). This method would only be suitable for small (S) fish.

The lack of sink garbage disposal units in Australian homes means that a blender is the only suitable means of maceration available to most ornamental fish owners. A kitchen blender that is used for food preparation is not considered a suitable tool for the euthanasia of sick fish. As with many of the physical methods of euthanasia, maceration is aesthetically unpleasant and may not be suitable for the retail environment or home owner. It is for this reason that it is included in this section.

4.4 Occupational Health and Safety

Many fish possess spines which, though generally not poisonous, may still cause unpleasant injuries. All fish handling should be done with this in mind and if the owner is handling a species that they are not completely familiar with, they should always be careful and seek advice from a more experienced person.

Spines may be located at several sites on fish –

- on the anterior (front) edge of fins including the dorsal (top) and pectoral (first side fin). Most catfish will have spines and even small species can cause an unpleasant injury. Many marine fish and especially members of the Scorpaenidae family possess nasty spines
- operculum (gill cover) these are seen in marine angelfish and anemonefish (Nemo)
- "blades" are found on the caudal peduncle (tail) area of some marine fish e.g. Tangs or Surgeon fish – family Acanthuridae.

Two fish groups which are very toxic are the lionfish (*Pterois spp.* and *Dendrochirus spp.*) and stonefish (*Synanceia spp.*) – members of the family Scorpaenidae. Handling these species should be done **VERY** carefully as the toxin from their dorsal spines is **EXTREMELY** painful. Only very experienced owners should handle these fish. The toxins of Scorpaenidae are heat-labile. This means that the toxin is readily destroyed by heat. Thus immersing the affected body region in water as hot as is bearable (without being scalding) will break down the toxin. This should be done as an immediate first aid measure and maintained on the way to hospital.

5. Recommendations

As discussed in Section 4.1 there is a wide variety of fish types that have different water requirements and are of differing sizes. These factors can have a significant impact on the methods of euthanasia that are used and that are considered humane.

With the various euthanasia techniques, whilst the salinity of the water is critical to avoiding an osmotic stress to the fish, this is easily prevented by placing the fish in water that is matched for salinity (a sample of the fishes tank water is ideal). Removing this factor and combining water temperature and size gives six possible combinations as listed in Table 6 with their abbreviations in parentheses

Table 6 - Abbreviations used to indicate different groups of fish

Water Type	**Size**	**Abbreviation**
Tropical	Small (< 5cm)	Tr S
	Medium (5 – 15cm)	Tr M
	Large (> 15cm)	Tr L
Temperate	Small (< 5cm)	Te S
	Medium (5 – 15cm)	Te M
	Large (> 15cm)	Te L

By reviewing all the available literature, the following techniques are considered humane –

- Clove oil is appropriate for all six groups providing that the fishes own water (or identical water parameters) is used and a volume of water appropriate to the fish size is used. However, there is not a registered product available in Australia.

- Aqui-S is appropriate for all six groups providing that the fishes own water (or identical water parameters) is used and a volume of water appropriate to the fish size is used. Aqui-S is registered and available in Australia.

- Decapitation, followed by pithing, is suitable for both small (S) and medium (M) fish (Tr S, Tr M, Te S and Te M).

- Percussive stunning, followed by pithing, is suitable for both medium (M) and large (L) fish (Tr M, Tr L, Te M and Te L).

- Rapid chilling / ice slurry is only suitable for Tr S fish.

This is summarized in the table below.

Table 7 – Summary of all possible humane euthanasia options

Abbreviation	**Acceptable Method**
Tropical Small	Aqui-S Rapid chilling / ice slurry Cervical decapitation, followed by pithing
Tropical Medium	Aqui-S Cervical decapitation, followed by pithing Percussive stunning, followed by pithing
Tropical Large	Aqui-S Percussive stunning, followed by pithing
Temperate Small	Aqui-S Cervical decapitation, followed by pithing
Temperate Medium	Aqui-S Cervical decapitation, followed by pithing Percussive stunning, followed by pithing
Temperate Large	Aqui-S Percussive stunning, followed by pithing

Most retail outlets and ornamental fish owners do not possess the ability or the dedicated space to conduct the physical methods of either decapitation or percussive stunning followed by pithing.

As such, until further research is conducted, this work recommends that the best and preferred method for the euthanasia of ornamental fish in the home and retail environment is the use of a registered clove oil derivative such as Aqui-S, as it covers all possible fish types. The availability in small volumes is an issue.

Glossary

Biotope – an area with uniform environmental conditions and the animal and plant life that reside in that environment

Elasmobranch – fish with a cartilaginous skeleton; includes sharks and rays

Grey literature – informally published reports or opinions; material not found in scientific journals

Hypothermal shock – shock associated with a sudden drop in temperature of the environment

Operculum – bony covering that protects the fish gills

Ornamental Fish – includes freshwater and marine teleosts (bony fish) and elasmobranchs (cartilaginous fish) that are kept at home either as aquarium or pond fish

Osmoregulation – the active control and regulation of the osmotic pressure of an organism's body fluids to maintain the homeostasis (normal balance) of the organism's water

Osmoregulatory – refers to osmoregulation

Osmotic stress – the stress associated with a sudden change in environmental salt levels relative to the internal salt level of an organism

Physiological – refers to the normal functioning of a living organism

Poikilothermic – the fishes internal body temperature is not maintained at a constant temperature but fluctuates depending on the temperature of the water in which they live

Teleost – fish with a bony skeleton; the majority of fish

Acronyms

AAWG - Aquatic Animal Working Group

AAWS - Australian Animal Welfare Strategy

ANZCCART - Australia and New Zealand Council for the Care of Animals in Research and Teaching

APVMA - Australian Pesticides and Veterinary Medicines Authority

AVMA - American Veterinary Medical Association

DA - Department of Agriculture

PIAA - Pet Industry Association of Australia

POCTAs - Prevention of Cruelty to Animals Acts

References

Appleby MC and Sandøe P, 2002, Philosophical debate on the nature of well-being: Implications for animal welfare, *Animal Welfare* 11: 283-294

Bagni M, Civitareale C, Priori A, Ballerini A, Finoia M, Brambilla G, and Marina G, 2007, Pre-slaughter crowding stress and killing procedures affecting quality and welfare in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). *Aquaculture* **263,** 52–60

Barker D, Allan GL, Rowland SJ and Pickles JM, 2002, A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research, 2nd Edition. *NSW Fisheries Animal Care and Ethics Committee*

Blessing JJ, Marshall JC and Balcombe SR, 2010, Humane killing of fishes for scientific research: a comparison of two methods. *Journal of Fish Biology* **76,** 2571–2577

Borski RJ and Hodson RG, 2003, Fish research and the institutional animal care and use committee. *Institute of Laboratory Animal Resources Journal* **44,** 286–294

Close B, Banister K, Baumans V, Bernoth EM, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth H, Morton D and Warwick C, 1996, Recommendations for euthanasia of experimental animals: Part 1. DGXI of the European Commission. *Laboratory Animals* **30,** 293–316

Close B, Banister K, Baumans V, Bernoth EM, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth H, Morton D and Warwick C, 1997, Recommendations for euthanasia of experimental animals: Part 2. DGXT of the European Commission. *Laboratory Animals* **31,** 1–32

DAFF, 2009, Operational procedures manual: Destruction (Version 2.0), pp 1–41

Danner RG, Muto KW, Zieba AM, Stillman CM, Seggio JA and Ahmad ST, 2011, Spearmint (l-carvone) oil and wintergreen (methyl salicylate) oil emulsion is an effective immersion anesthetic of fishes. *Journal of Fish and Wildlife Management* **2,** 146–155

FishBase Available at < http://www.fishbase.org> Viewed June 2013

Gerlai R, Lahav M, Guo S and Rosenthal A, 2000, Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. *Pharmacology, Biochemistry and Behaviour* **67,** 773–782

Håstein T, Scarfe AD and Lund VL, 2005, Science-based assessment of welfare: aquatic animals, Scientific and Technical Review of the Office International des Epizooties (OIE) 24 (2), pp529-547, <http://www.velferdsprotokoller.org/uploads/5/4/3/3/5433758/science-based_assessment_of_welfare_-_aquatic_animals.pdf > Viewed June 2013.

Holloway AC, Keene JL, Noakes DG and Moccia RD, 2004, Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. *Aquaculture Research* **35,** 1025–1030

Lambooij B, Gerritzen MA, Reimert H, Burggraaf D, Andre G, and Van de Vis H, 2007, Evaluation of electrical stunning of sea bass (*Dicentrarchus labrax*) in seawater and killing by chilling: welfare aspects, product quality and possibilities for implementation. *Aquaculture Research* **39,** 50–58

Leary S, Underwood W, Anthony R, Cartner S and Corey D, 2013, AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. American Veterinary Medical Association, Schaumberg IL, USA

Matthews M and Varga ZM, 2012, Anesthesia and euthanasia in zebrafish. *Institute of Laboratory Animal Resources Journal* **53,** 192–204

Morzel M, Sohier D and Van De Vis H, 2003, Evaluation of slaughtering methods for turbot with respect to animal welfare and flesh quality. *Journal of the Science of Food and Agriculture* **83,** 19–28

Oxford Dictionary. <http://oxforddictionaries.com/definition/english/humane> Viewed June 2013

Panaquatic, 2006, *A Review of Current Welfare Arrangements for Finfish in Australia* <http://www.daff.gov.au/__data/assets/pdf_file/0017/152108/aaws_stocktake_aquatic.pdf> Viewed June 2013

Peng J, Wagle M, Mueller T, Mathur P, Lockwood BL, Bretaud S and Guo S, 2009, Ethanol-modulated camouflage response screen in zebrafish uncovers a novel role for cAMP and extracellular signal-regulated kinase signaling in behavioral sensitivity to ethanol. *Journal of Neuroscience* **29,** 8408–8418

Poli BM, Parisi G, Scappini F and Zampacavallo G, 2005, Fish welfare and quality as affected by pre-slaughter and slaughter management Peer reviewed article. *Aquaculture International* **13,** 29–49

Rahmanifarah K, Shabanpour B and Sattari A, 2011, Effects of clove oil on behavior and flesh quality of common carp (*Cyprinus carpio L.*) in comparison with pre-slaughter CO_2 stunning, chilling and asphyxia. *Turkish Journal of Fisheries and Aquatic Sciences* 11: 139-147

Reilly JS, 2001, *Euthanasia of animals used for scientific purposes*, Second Edition, Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART) Adelaide University, Adelaide SA, Australia

Roth B, Imsland AK and Foss A, 2009, Live chilling of turbot and subsequent effect on behaviour, muscle stiffness, muscle quality, blood gases and chemistry. *Animal Welfare* **18,** 33–41

Savage J, 2013, *Status of Australian Aquaculture in 2010/11*, Austasia Aquaculture Trade Directory 2013, Turtle Press, Hobart, Tasmania, Australia

Topic Popovic N, Strunjak-Perovic I, Coz-Rakovac R, Barisic J, Jadan M, Persin Berakovic A, and Sauerborn Klobucar R, 2012, Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. *Journal of Applied Ichthyology* **28,** 553–564

Van de Vis H, Kestin S, Robb D, Oehlenschlager J, Lambooij B, Munkner W, Kuhlmann H, Kloosterboer K, Tejada M and Huidobro A, 2003, Is humane slaughter of fish possible for industry? *Aquaculture Research* **34,** 211–220

Wilson JM, Bunte RM and Carty AJ, 2009, Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). Journal of the American Association for Laboratory Animal Science **48,** 785–789

Appendix One

AAWS Aquatic Animal Working Group – Overarching Principles

In the context of Aquatic Sector of the Aquatic Animal Welfare Working Group under the Australian Animal Welfare Strategy (AAWS), only vertebrate finfish are considered Aquatic Animals; other aquatic vertebrates are considered under other Sectors of AAWS. ***(Note 1)***

The approach taken with animal welfare to date within the Aquatic Animal sector has been to establish overarching Principles against which sub-sectors can build their specific best practice guidelines to achieve animal welfare. ***(Note 2)***

The overall aim of the aquatic sector (fish that are farmed, being transported, kept in aquaria, captured from the wild both commercial and recreational, or in aquaria in restaurants) should be to minimise suffering within the constraint of practices inherent to that sub-sector. ***(Note 3)***

Specific measures include:

1. For fish held in captivity, the key parameters (temperature, salinity, pH, dissolved oxygen, & metabolites) of the aquatic environment in which fish are maintained should be within the species' natural range of tolerance.

2. For fish held in captivity, the holding unit in which they are normally housed should provide
    - safety from predators,
    - refuge from environmental extremes beyond their natural range of tolerance,
    - appropriate space,
    - appropriate space and/or water flow to avoid chronic degradation of water quality parameters referred to in point 1 above. ***(Note 4)***

3. For fish held in captivity the feed supplied should meet known nutritional requirements, and be distributed in a manner and frequency which avoids starvation for periods longer than the species natural range of tolerance.

4. For fish held in captivity, any visibly damaged or sick fish should be assessed and either treated appropriately or promptly removed for killing by humane means suitable for the species.

5. During any handling of live fish,
    - care should be taken to avoid any damage to the fish

- for prolonged handling of fish out of water (e.g. health checks, vet treatment, artificial reproduction, etc), an anaesthetic appropriate for the species and frequent irrigation of skin and gills is essential
- fish intended to remain alive should be returned to the water promptly.

6. Any fish selected for harvest should be killed as rapidly as possible, by humane means suitable for the species

7. For fish harvested from the wild timely handling from capture to death is essential to minimise suffering. ***(Note 5)***

8. Capture methods should be designed to minimise the capture of unwanted fish.

Explanatory Notes

Note 1: The duty of care principles are couched within the Australian Animal Welfare Strategy under which these specific aquatic animal principles will be applied.

Note 2: As a code there is no legislative basis. Words such as 'must' hold no relevance. Animal Welfare legislation is the place for definitives and the code assists operators to meet those definitives through words such as 'should'.

Note 3: Suffering is inclusive of pain and other issues of animal welfare.

Note 4: This principle when read with principle 1 covers all aspects. The detail of parameters such as water flow, stocking density, behavioural aspects and space will be in the sub-sector code themselves depending on operational method and species.

Note 5: 'Capture' as defined in sub-sector codes.

Appendix Two

Steps to Performing Humane Euthanasia using Rapid Chilling / Ice Slurry

1. This technique is ONLY suitable for Small fish (< 5cm) that are also tropical (>22^{o}C).

2. Prepare a container of the correct size for a small (S) fish = 2 litres (e.g. an old ice-cream container).

3. Fill the container to three-quarters (3/4) full with cube or crushed ice (Figure 6). Note that ice made from freshwater is suitable for marine fish.

4. Fill the container to the level of the ice with water from the tank that the fish was kept in (Figure 7). The mixture should contain a ratio of at least 1:1 ice: water.

5. Wait ten minutes for the temperature to stabilize and then make a depression in the ice slurry where there is water only to place the fish to be euthanized (Figure 8).

6. Gently transfer the fish to the prepared container with a net and place it in the depression. At all steps gentle handling of the fish is essential.

7. Operculum movements should cease within one to two minutes. Leave the fish in the solution for an absolute minimum of one hour.

8. Remove the fish and dispose of the body in the correct manner (refer to Section 2).

9. Water used for euthanasia should ideally be placed on the garden, but may also be flushed down the toilet.

Ice water slurry prepared in a 2-L ice cream container as described above will reach a temperature of approximately 1.5 - 2.0ºC after 10 minutes, and will remain at that temperature for up to 60 minutes (personal observation).

If the operator does not wish to wait one hour then after ten (10) minutes it is possible to remove the fish from the ice slurry and pith it as detailed above (a two-step process).

Figure 6 – A two-litre ice cream container approximately three-quarters full of ice.

Figure 7 – Ice slurry made by adding tank water to the ice cubes in a two-litre ice cream container.

Figure 8 – Euthanasia of a small (<5cm) tropical fish in an ice slurry.
(Please note this is a plastic fish and no live fish were used in this demonstration)