Stunning and Killing of Tropical and Subtropical Finfish in Aquaculture during Slaughter

Jennifer Bowman & Albin Gräns

DEN 25 FEBRUARI 2019

Contents

1 Sammanfattning på Svenska

I dag får ca 3 miljarder människor en stor del sitt dagliga intag av protein från fisk och under de senaste 70 åren har världens fiskkonsumtion mer än sexfaldigast. Denna enorma ökning har, i en tid då 90 % av de kommersiellt viktiga fiskbestånden utarmats, möjliggjorts genom ökad produktion av matfisk i fångenskap. Tyvärr finns det också problem kopplade till vattenbruk och välfärden för odlad fisk är ur många aspekter undermålig. Detta kan delvis beropå att det är svårt att bedöma fiskars välfärd eftersom fiskars beteenden som signalerar obehag, sjukdom och smärta inte liknar de som ses hos andra djurslag och delvis på att fiskar lever i en miljö där vi har svårare att observera dem. För att i framtiden kunna uppnå ett hållbart vattenbruk och sedan ytterligare expandera detta för att möta världens ökande behov av fiskprotein, måste all utveckling åtföljas av vetenskapliga studier ämnade att minimera stressen i odling, samt garantera att fisken avlivas snabbt, smärtfritt och effektivt.

I den här rapporten har vi sammanställt befintlig litteratur, publicerad i vetenskapliga tidskrifter, med avseende på stress och djurvälfärd vid bedövning, avlivning och slakt av fiskarter som definieras som tropiska eller subtropiska. 2016 odlades minst 149 arter (se Appendix) som innefattas i dessa grupper men även om de tropiska eller subtropiska arterna dominerar världens fiskodlingar så utgör informationen i rapporten ett mycket begränsat underlag då vi endast fann studier rörande 18 av dessa 149 arterna. Ett annat återkommande problem i vår sammanställning är svårigheten att bedöma fiskar medvetande med hjälp av endast visuella tecken. I ett flertal olika studier där man bedömt fiskars grad av medvetande med mätningar av hjärnaktivitet genom Elektroencefalografi (EEG) har man påvisat att fiskar kan förlora alla visuella tecken på medvetande långt före hjärnaktiviteten har försvunnit. I värsta fall betyder det att fiskar som ser bedövad ut endast paralyserats och i själva verket riskerar att uppleva bedövningen och/eller avlivningen. I rapporten har vi valt att inkludera även studier som endast undersökt visuella tecken på medvetande då resultaten från dessa studier kan användas för att identifiera när en undersökt metoder inte uppfyller kraven på en etiska försvarbar avlivningsprocedur. Nedan följer en sammanfattning av de viktigaste resultaten för respektive metod för bedövning, avlivning och slakt av tropiska eller subtropiska fiskar som inkluderats i rapporten.

Förblödning och/eller kvävning i luft

De två vanligaste metoderna för att avliva fisk är genom kvävning i luft eller genom någon form av förblödning. Om detta sker utan någon form av bedövning så innebär det en lång och stressfull död för alla undersökta arter.

Bedövning i vatten mättat med gas

Bedövning med koldioxid är även det en utdragen process som för de allra flesta undersökta arter var kopplade till negativa beteenden och kraftigt förhöjda stressnivåer. Undantaget här var en studie på Niltilapia (*Oreochromis niloticus*) där det förvisso tog hela 30 min för djuren att förlora alla visuella tecken på medvetande men i studien noterades inga negativa beteenden och efter bedövningen var stressnivåerna fortfarande på nivå med ett ostressat djur. Även i en annan studie på Niltilapia där kolidoxiden bytts ut mot kväveoxid tog det lång tid (20 min) för djuren att förlora alla visuella tecken på medvetande men återigen noterades inga negativa beteenden. I detta fall visade dock fiskarna som genomgått kväveoxid-bedövningen kraftigt förhöjda stressnivåer.

Bedövning genom nedkylning

Nedkylning sker vanligtvis i ett isbad eller i kraftigt nedkylt vatten där temperaturen ligger på endast ett fåtal grader. Även nedkylning är en utdragen process som för de allra flesta undersökta arter var kopplade till negativa beteenden och kraftigt förhöjda stressnivåer. Återigen så skiljer sig Niltilapia från de andra undersökta arterna genom att de efter 20 min nedkylning fortfarande hade stressnivåerna på nivå med ett ostressat djur. Även en studie utförd på arten Senegaltunga (*Solea senegalensis*) rapporterade ett förhållandevis lågt stresspåslag i fiskar som flyttats från 16°C till 2-4°C kallt vatten tills det att de förlorat visuella tecken på medvetande (tiden det tog rapporterades inte).

Bedövning med slag eller syl

Ett flertal studier på olika fiskarter har visat att både slag och syl kan vara ett snabbt och effektivt sätt att bedöva/avliva fisk. Medan effekten av ett korrekt utfört slag är momentan så tar det 10-20 s för en syl-behandling att verka. Effekten av båda metoderna är dock bestående. Det finns dock uppenbara problem med att både lyckas med utförandet samt att med hjälp av visuella tecken verifiera att behandlingen lyckats utföra dessa bedövningsmetoder. Till exempel så vet vi att ett slag i huvudet kan paralysera en fisk utan att den förlorar medvetandet. Det har också visat sig i både laboratorier studier och studier som görs på plats ute i industrin att andelen lyckade utföranden varier kraftigt mellan studier.

Elbedövning

Även elbedövning kan vara ett snabbt och effektivt sätt att bedöva fisk. Oftast är effekten av en korrekt utförd elbedövning momentan men i de allra flesta fall är effekten övergående och i vissa studier har fiskarna återfått medvetandet inom 30 s vilket betyder att det måste efterföljas av en snabb och effektiv avlivningsmetod. Då detta sällan ingår i de vetenskapliga studierna är det svårt att avgöra hur användbara de olika studiernas resultat är i praktiken. Ett undantag är en studie på karp (*Cyprinus carpio*) där man efter en effektiv elbedövning flyttade fiskarna till ett isbad och på så sätt motverkade att fiskarna återfick medvetandet. Ett liknade försöksupplägg användes i en studie på sjötunga (*Solea solea*) men i den studien hjälpte inte isbadet utan fiskarna återfick medvetandet kort efter elbedövningen. Ett annat problem är att det finns ingen följdriktighet mellan studier. Elbedövning är tekniskt komplicerat på en helt annan nivå än de andra beskrivna metoderna och vilken typ av elchock som de maskiner som idag säljs kommersiellt använder sig av skyddas till stor grad av företagshemligheter.

Bedövning med essens från kryddnejlika

I många länder utanför Europa och USA är det vanligt att använder essens från kryddnejlika för att bedöva fisk innan avlivning. En rad studier på en flera olika arter har visat att med rätt dos kan man på 1-25 min bedöva fisk utan några negativa beteenden och med stressnivåer på nivå med ett ostressat djur. All befintliga studier använder sig dock av visuella tecken för att utvärdera fiskarna grad av medvetande vilket medför att kunskapen om drogens effekt på fiskarnas hjärnaktivitet är fortfarande låg.

2 Introduction

Today, aquaculture is one of the fastest growing food production sectors. In 2016, the world aquaculture production of fish, crustaceans, molluscs and aquatic plants for human consumption was 110.2 million tonnes, with finfish comprising nearly 50% (54.1 million tonnes) of production (FAO, 2018b). There is increasing pressure to provide a safe, healthy and nutritious food source for the growing population, and fish has excellent potential to be the solution. Fish are already a staple in many countries and are an efficient source of proteins and omega-3 fatty acids.

While there is focus on meeting the needs of the growing population, the welfare of fish is often overlooked. There is an incredible amount of research devoted to the product quality of fish produced in aquaculture and how the harvesting process affects fillets, but very few that take welfare into account. This is especially true with regards to tropical and subtropical fish, where the most harvested species contribute to more than 60% of the total finfish production (34.4 million tonnes), with estimated numbers between 26.3 and 94.3 billion being harvested each year (see Table 1).

The harvesting process is especially stressful for fish. From capture to stunning, all factors should be examined in order to ensure that fish are being handled and processed in the most humane way possible.

2.1 Animal Welfare

2.1.1 What is welfare?

The OIE (World Organization for Animal Health) has defined animal welfare with regards to all species of animals farmed for food.

> *"Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behavior and it is not suffering from unpleasant states such as pain, fear and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter / killing." (OIE, 2018)*

Although this definition should also be applied to fish there has been little scientific research on the conditions of fish stunning and killing during slaughter in aquaculture. With the incredibly high number of individuals affected in fish farming, in general and for tropical and subtropical species in particular (see Table 1), it is imperative that additional research be conducted to ensure the welfare of fish is maintained at an optimal level at all points during their life including the harvesting process.

2.1.2 Stress and visual indicators of consciousness

The harvesting process involves a number of different stresses for fish, including feed withdrawal, capture, crowding, handling, loading and unloading, transport, and preparation for the stunning procedure. Stress can be seen in movements, including escape attempts, flaring of the operculum, struggling to maintain righting reflex (equilibrium), and aversive behavior (Poli et al., 2005; Van de Vis et al., 2003). However, stress response is not limited to visible behavior changes. When fish are stressed there is a measurable physiological stress response including the release of catecholamines and corticosteroids into the blood stream (Barton & Iwama, 1991). Cortisol is the main corticosteroid

seen in teleosts and how much is released is dependent heavily on the species of fish and the duration and severity of the stressor (Barton & Iwama, 1991).

In order to minimize the stress perceived during killing and slaughter it is critical that the animal is stunned immediately prior and remains unconscious until death. However, unconsciousness in fish can be difficult to determine, and both scientists and fish farm personnel commonly rely on visual indicators to determine the conscious state of fish. Commonly used visual indicators of consciousness include maintenance of equilibrium, ventilation, response to stimulation, and the eye-roll reflex. With the eye-roll reflex, the fish is tilted from side to side and if the eyes adjust to compensate for the tilt then the fish is still conscious (Kestin et al., 2002; Robb & Kestin, 2002). Eye-roll reflex and ventilation have been used as semi-reliable indicators of consciousness, as these are reported to be the last responses lost during anesthesia and the first to return upon waking (Kestin et al., 2002). A more accurate method of assessing brain activity is the application of electroencephalography (EEG), and a number of researchers have used EEG in combination with visual indicators to determine when consciousness is lost. This is especially important as an incorrectly executed stun can render fish paralyzed and neither insensible nor unconscious, which would go unknown through visual assessment alone but would be seen using EEG.

2.1.3 Electroencephalography and visual evoked responses

Electroencephalography (EEG) can be used to determine when a fish transitions to a state of unconsciousness through analysis of changes in brain activity (Kestin et al., 1991; Kestin et al., 2002; Grimsbø, 2016). EEG measures brain activity through the surgical implantation of electrodes or through contact with the skin (Quick & Laming, 1990; Kestin et al., 1991; Kestin et al., 2002). EEG allows for the detection of visual evoked responses (VERs), a popular method of assessing consciousness in both humans and other animals including fish (Kestin et al., 1991; Kestin et al., 2002). The most common method to measure VERs in fish is by stimulation from a flashing light, which produces a distinct waveform on EEG recordings (see Figure 1). As the fish transitions from a conscious to unconscious state the waveform decreases and once response is gone (i.e. the fish is no longer responsive to its surroundings) the fish is assumed unconscious (Robb et al., 2000).

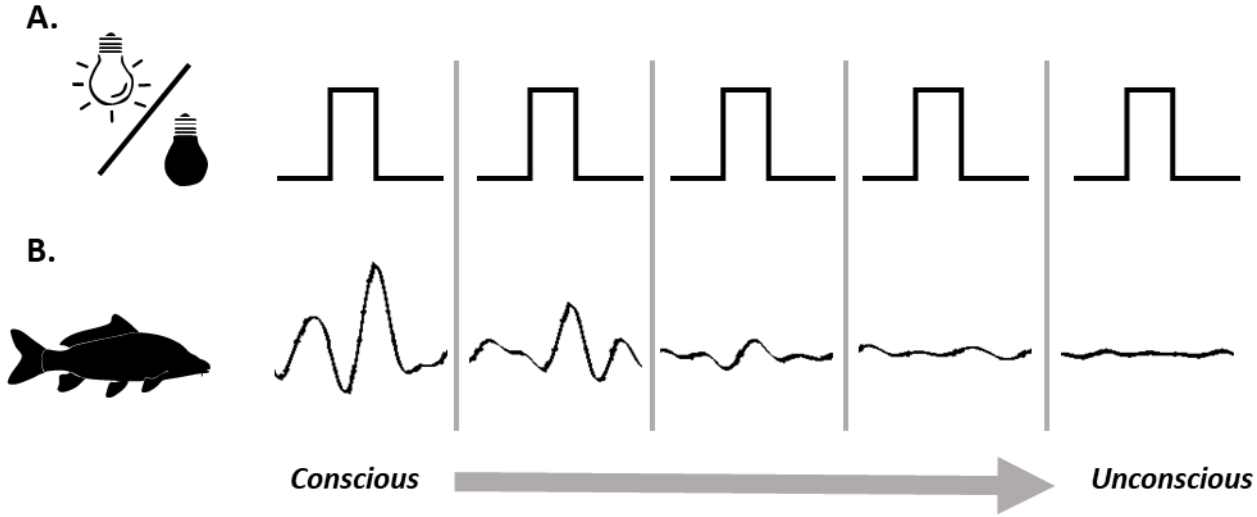

Figure 1: An example of visual evoked responses (VERs) on an EEG recording a fish while losing consciousness. A) Shows the stimulus of the flashing light (2Hz) and B) shows the average of 120 consecutive VERs registered on the EEG.

2.2 Stunning methods

Ideally, stunning should be quick and easy to administer and leave the animal unconscious until death (Ross et al., 2009). Unfortunately, not all stunning and killing practices are ideal for all fish, and as a result there is a great need for scientific assessment of what methods best ensure welfare for different species. Below follows a brief description of the different methods used to stun, kill, and slaughter tropical and subtropical fish as described in the available literature. For a more comprehensive description of the different methods used to kill fish, see Robb and Kestin (2002) and Van de Vis et al. (2003).

2.2.1 Percussion and spiking

Percussive stunning is where fish are hit on the head using manual force with a rapidly moving club, also called a priest, or with a bolt using automatic force. When administered correctly, a manual percussive stun will instantly render a fish unconscious but requires training and precision for those applying it (Robb et al., 2000). However, when applied manually it can be difficult to determine how much force is required to induce the desired state, and because fish are stunned individually it means adjusting the force on a fish-by-fish basis. The force required to stun fish may cause injuries, such as broken jaws and hemorrhaging and burst eyes, while not enough force can mean the fish is not rendered unconscious (Kestin et al., 1995; Lambooij et al., 2010; Poli et al., 2005; Robb et al., 2000).

Automated stunning machines can be used to process larger quantities of fish in quicker succession. However, most currently available machines are limited to fish of a certain size, and fish that are either too large, small or deformed may not be stunned correctly (Grimsbø, 2016).Spiking involves inserting a spike into the brain and using rotation to ensure the brain is destroyed. This method can be done manually or automatically with a pressurized stun gun (Robb et al., 2000; Robb & Kestin, 2002). When correctly applied, spiking immediately causes loss of consciousness. However, spiking has same force problems as percussion, where not enough force can cause injury and require additional applications to achieve the desired state (Robb et al., 2000; Robb & Kestin, 2002). Successful spiking can also be difficult as a high level of accuracy is needed to hit the brain and achieve the desired result (Lambooij et al., 2010)

2.2.2 Electrical

Electrical stunning is the process of passing an electrical current through a fish while submerged in a water bath (wet electrical stunning) or while on an electrified grid exposed to air (dry electrical stunning) (Gräns et al., 2016; Kestin et al., 2002). This process is quick and inexpensive, but is a delicate operation. When the correct current is used, fish instantly lose visual indicators of consciousness and movement. However, if incorrect or insufficient current are used the fish can exhibit strong negative responses as soon as the current is turned off (Kestin et al., 1995). In some cases, incorrect electrical stunning can render a fish paralyzed but not unconscious (Readman, 2015; Van de Vis et al., 2003). This state is normally referred to as electro-immobilization and is especially concerning as fish are unable to display aversive behavior while in this state, making it impossible to gauge the success of the stun (Van de Vis et al., 2003).

Electrical stunning is the method most commonly paired with EEG analysis. Unconsciousness with this method is characterized by an epileptiform insult on the EEG recording, indicating the fish has experienced seizure activity, and loss of VERs. However, stunning with this method usually

produces a short window at which fish are unconscious before visual indicators return, leaving a short time period for humane slaughter as the fish risk recovery prior to death (Van de Vis et al., 2003).

2.2.3 Carbon dioxide (CO_2)

Carbon dioxide (CO_2) stunning involves the saturation of water with CO_2 until pH reaches <5 indicating water is fully saturated (Anonymous, 1995). CO_2 dissolves into the bloodstream and causes CO_2 narcosis, a systemic pH drop, oxygen deficiency, and respiratory failure (Kaiser & Lien, 2006; Kugino et al., 2016). While this process allows for the stunning of large groups of fish in a relatively easy and inexpensive manner, it is not without fault. CO_2 stunning has been cited as aversive for a number of fish and some studies have shown that unconsciousness does not occur immediately (Robb & Kestin, 2002; Robb et al., 2000). CO_2 narcosis can also cause physical changes to the fish as well with increased mucus production, making the fish harder to handle post stunning (Gräns et al., 2016; Marx et al., 1997; Robb & Kestin, 2002).

2.2.4 Clove oil

Clove oil is distilled from the clove tree (*Syzygium aromaticum*) and contains a high percentage (up to 90%) of the active ingredient eugenol (4-allyl-2-methoxyphenol) (Anderson et al., 1997). Clove oil is rapidly absorbed through the gills and causes decreased respiratory rates and ultimately death (Hikasa et al., 1986). The addition of clove oil mixed with ethanol to water can be used as an easy and inexpensive method to stun groups of fish immediately before killing and slaughter, a method normally referred to as rested harvest (Bosworth et al., 2007). The use of clove oil as a stunning method for food fish is still under investigation for safety with regards to human consumption in the EU and the United States (Robb & Kestin, 2002) but is approved under the name AQUI-S in Australia, Chile, New Zealand, Korea, Costa Rica and Honduras with a zero holding period prior to eating (Aqui-s.com, 2018).

2.2.5 Live/ice chilling

Immersing fish in a slurry mixture of ice and water, often referred to as hypothermia, is a commonly used stunning technique for fish. The immersion causes cold shock, rapidly decreasing the body temperature of fish and resulting in the loss of visual indicators of consciousness. While this method is known for being beneficial to product quality, ice chilling risks immobilization of fish without causing unconsciousness (Robb & Kestin, 2002). Due to the possibility of immobilization, it is difficult to determine if fish find this process aversive and time to loss of consciousness according to visual indicators may be inaccurate (Robb & Kestin, 2002).

2.3 Killing methods

2.3.1 Asphyxiation in air

Asphyxiation in air is the process by which a fish is removed from water and exposed to air for an extended period of time causing the fish to suffocate. This method often requires a long exposure time before visual indicators are lost, during which fish exhibit extremely aversive behavior (Acerete et al., 2009; Bagni et al., 2007; Rahmanifarah et al., 2011; Van de Vis et al., 2003).

2.3.2 Exsanguination through gill cutting, decapitation or evisceration

Gill cutting is a commercial slaughter method in which fish are restrained and a knife is used to cut the gill arches on one or both sides of the throat. The gill cutting causes the fish to bleed out, either while exposed to air or submerged in an ice slurry (Robb & Kestin, 2002). When done in air, fish die of a combination of exsanguination and asphyxiation.

Decapitation is the severing of the head from the body (Robb & Kestin, 2002). This method is commonly used for eels but is unsuitable as a killing method for other fish as application can be difficult (Robb & Kestin, 2002; Verheijen & Flight, 1997).

Evisceration is the removal of the liver and intestine alone or in combination with the heart. The cause of death is usually a combination of exsanguination and asphyxiation (Robb & Kestin, 2002). Evisceration is common practice during processing of wild caught fish but also occurs on fish farms that do not employ any means of stunning prior to killing.

2.4 The effect of temperature

Temperature plays an important role in the efficacy of stunning, killing and slaughter, which is especially important to consider when stunning fish in warmer tropical and subtropical climates. Brain activity and visual indicators of consciousness, such as ventilation, can be lost faster at warmer temperatures. This is because metabolic rate is dependent on temperature, and oxygen supplies are consumed faster at higher temperatures (Kestin et al., 1991; Robb & Kestin, 2002). Temperature of the water also affects how much and how quickly different solutions are dissolved in. This is important when using anesthetics or carbon dioxide as a stunning method as warmer temperatures can increase the rate of induction (Ross et al., 2009; Santos et al., 2015; Sneddon, 2003; Topic Popovic et al., 2012). Additionally, submersion in an ice slurry as a stunning practice will be more effective when there is a large temperature difference. Electrical stunning is also affected by temperature, as electrical conductivity of water increases by 2-3% with each increase of 1ºC (Matthess, 1982; Hayashi, 2003 [Sources unrelated to stunning of fish]).

3 Fish

Fish with a habitat range that overlapped the subtropical range by more than 5° are included in this report. The subtropical latitude range is loosely defined as 35° north and south of the equator (*Subtropics - AMS Glossary*, 2012), while tropical is defined as the range between the tropic of Cancer in the Northern hemisphere (23.43683°N) and the tropic of Capricorn (23.43683°S) in the Southern hemisphere (Sitnikov, 2009). According to our investigations, using statistics provided by the FAO on aquaculture in 2016, there are currently 167 different fish species farmed in aquaculture with a habitat within the subtropical or tropical range (determined using www.fishbase.de). Of these fish, no literature concerning welfare during stunning, killing or slaughter was found for 149 (see appendix 1), and as such only 18 species are included in this review. Worth noting is that out of the 19 tropical and subtropical species with a production volume exceeding 350 000 tonnes in 2016 (see Table 1) we found information for just 5 of these species.

Fish in this report are described in order of tonnes harvested for aquaculture in 2016. Statistics on global aquaculture production were obtained from the FAO Fishery statistics collection (http://www.fao.org/fishery/statistics/global-aquaculture-production/en). For a full summary of all stunning methods and species reviewed in this report see Table 2.

3.1 Grass carp (*Ctenopharyngodon idellus*)

6 068 015 tonnes of grass carp were farmed for aquaculture in 2016. This fish has a latitudinal range of 65°N - 25°N and are tolerant of temperatures up to 38°C.

A study by Scherer et al. (2005) examined the effect of both ice-water slurry and electrical stunning as killing methods on grass carp stored on ice. Fish were randomly selected to be immersed in a 1:1.5 ratio of ice to water slurry for 20 min, or killed using direct current between two submerged copper plate electrodes over the course of 3 min (3 A / 200 V for 1 min followed by 3.5 A / 220 V for 2 min). Fish killed by ice-slurry ceased movements 10 min after transfer, with strong aversive behavior during the first min. After 20 min of immersion fish were deemed dead. Grass carp killed using direct current stopped moving immediately once the current began (Scherer et al., 2005). No information regarding the state of the carp when the current ended was reported.

3.2 Silver carp (*Hypophthalymichthys molitrix*)

The silver carp has a wide latitudinal range between 64°N - 43°S. 5 300 736 tonnes of silver carp were farmed for aquaculture in 2016.

In a study by Zhang et al. (2017), 10 trained panelists analyzed the behavior of 16 silver carp before manual percussive stunning using a wooden club. Before stunning, carp displayed normal swimming behavior, maintained equilibrium, had regular ventilation and exhibited a normal escape reaction, all of which ceased immediately after percussive stunning. A much slower transition to unconsciousness occurred when 16 silver carp were placed in a 1:1 ice:water slurry (Zhang et al., 2017). Fish transitioned from normal to decreased swimming attempts, minor escape attempts and irregular ventilation as their body temperature cooled. After 50 min, fish were deemed unconscious through visual observation of the panelists (Zhang et al., 2017).

After gill cutting, silver carp experienced severe bleeding but displayed no escape behavior and had decreasing ventilation until they were visually deemed unconscious after 40 min (Zhang et al., 2017). However, in a later study by the same research group it was noted that silver carp displayed severe struggling attempts and aversive behaviors during gill cutting (Zhang et al., 2018).

3.3 Common/European carp (*Cyprinus carpio*)

In 2016, 4 556 622 tonnes of common carp were farmed for aquaculture. Common carp are able to tolerate temperatures of 3°C to 35 °C and have a moderately wide latitudinal range of 60°N - 22°N.

In a field study by Retter et al. (2018), visual signs of consciousness and measurements of plasma cortisol were used to investigate the stunning efficiency of manual percussion, wet or dry electrical stunning, and electrical stunning followed by manual percussion. They found that found that 46.2% of common carp showed visual signs of consciousness at the time of slaughter after being stunned using manual percussion (Retter et al., 2018). The difficulties of ensuring percussive success with common carp was seen in the average blood cortisol level, which was 228 ng/ml after percussion, compared to levels between 5 and 15 ng/ml reported for unstressed carp (Retter et al., 2018). Elevated plasma levels of cortisol in common carp stunned using manual percussion were also previously shown in studies by Daskalova et al. (2016b) and Varga et al. (2014).

When electrical stunning was used (3 instances of wet electrical, 1 instance of dry electrical), 28.1% of common carp showed signs of consciousness at the time of slaughter (Retter et al., 2018). Fish stunned using this method had an average plasma cortisol level of 114 ng/ml, indicating that the

process was stressful (Retter et al., 2018). In an earlier study by Daskalova et al. (2016b), 7 electrically stunned (300V / 4.7 mA DC for 3 s) common carp had conflicting cortisol concentrations, with 2 carp having low levels of 3.21 and 13.80 ng/ml, while 1 fish had 603.93 ng/ml, and 4 carp had concentrations well above 800 ng/ml.

When electrical stunning (8 instances of wet electrical, 2 instances of dry electrical) was followed by percussion, 11.8% of the common carp showed visual signs of consciousness at the time of slaughter and mean cortisol levels were 151 ng/ml (Retter et al., 2018). From their results, Retter et al. (2018) concluded that both percussive and electrical stunning may be a poor inducers of unconsciousness before killing common carp (Retter et al., 2018). Although electrical stunning followed by percussion was the most effective method in the study it did not universally ensure the welfare of the fish. Success of the stun depended heavily on water conductivity, current duration, the voltage and current used and whether the stun was wet or dry. In one instance of wet electrical stunning followed by percussion, 66.0% of the common carp showed visual signs of consciousness at the time of slaughter (Retter et al., 2018).

The same study measured brain activity in common carp fitted with EEG electrodes during wet electrical stunning in a lab setting. When carp were stunned between two plates positioned top/bottom or laterally for 1 to 5 min at current densities between 0.09 and 0.4 A/dm^2 and electrical field strength between 15.5 and 68.2 V/dm they showed no visual indicators of consciousness after the electrical current ceased. However, in 31 out of 32 fish tested, brain activity returned within 30 s after stunning, long before visual indicators of consciousness were observed (Retter et al., 2018).

When 22 EEG equipped carp were subjected to wet electrical stunning in fresh water, a current of 113 V / 50 Hz AC for 1 s produced an effective stun that lasted 34 ± 10 s (Lambooij et al., 2007). 12 carps responded to needle scratches to the dorsal skin (pain stimuli) 30 s post stun, 20 after 2 min, and after 10 min all 22 carp responded to pain stimuli (Lambooij et al., 2007). When EEG equipped carp were restrained and stunned using whole body electrical stunning (411 V / 50 Hz AC) for 5 s in fresh water followed by chilling in ice water, it was found to be an effective slaughter method. 22 ± 13 s after sunning, EEG recordings showed minimal brain activity and fish did not respond to pain stimuli (Lambooij et al., 2007).

Head-only electrical stunning using tongs to deliver a current of 163 V / 50 Hz AC for 1 s was used to stun EEG fitted common carp effectively (Lambooij et al., 2007). Seizure activity, indicative of unconsciousness, was immediately registered on EEG recordings for all 10 carp stunned and lasted for 31 ± 14 s after stunning ceased. An additional 10 fish, unequipped with EEG, were stunned using the same current and showed visual indicators of consciousness 48 ± 8 s after the electrical current was turned off. Swimming resumed 121 ± 83 s after stunning ceased (Lambooij et al., 2007).

Lambooij et al. (2007) stunned EEG equipped carp (n=22) using captive bolt stunning with 7.5 bars of pressure, This method immediately produced minimal brain waves, indicative of unconsciousness and no brain activity was seen in 18 of 20 fish 16 ± 12 s after stunning. The force of the percussion caused EEG electrodes to disconnect in 2 fish (Lambooij et al., 2007). Rahmanifarah et al. (2011) transferred 8 carp to a tank containing a solution of water and 1 ml/L clove oil. Equilibrium was lost 65 ± 6.3 s after transfer and opercular movement ceased after 225 ± 24 s. Carp demonstrated no aversive behavior during this time and were noted to appear "calm" throughout treatment (Rahmanifarah et al., 2011).

Common carp transferred to an ice slurry (0.6-1.8°C) were observed swimming slowly for 11 min after submersion followed by aversive swimming, tremors, and escape attempts for the next 7 min, after which no aversive behavior was seen (Rahmanifarah et al., 2011). Opercular movement

ceased after an average of 48 min from the beginning of treatment. In a different study, it was shown that when carp were stunned in an ice slurry the blood cortisol increased to an average concentration of 275 ng/ml (Varga et al., 2014), much higher than that of unstressed carp reported in other studies (Retter et al., 2018).

When transferred to a tank containing water with dissolved CO_2 carp immediately showed aversive behavior (Rahmanifarah et al., 2011). All 8 carp tested made numerous escape attempts and continued aversive swimming even after equilibrium was lost 3 min after submersion. Aversive behavior continued for 7 min after transfer. While carp did not respond to physical stimulation after 8 min in the tank, opercular movement continued for 10 min after immersion (Rahmanifarah et al., 2011). Varga et al. (2014) found that carp submerged in water saturated with carbon dioxide had an average blood cortisol concentration of 200 ng/ml, much higher than unstressed concentrations.

When asphyxiated in air, common carp showed significantly higher cortisol levels compared to unstressed fish, with only 1 carp of 7 having a cortisol level less than 800 ng/ml (633.93 ng/ml) (Daskalova et al., 2016b). Common carp asphyxiated in a study by Rahmanifarah et al. (2011) showed violent aversive behavior when exposed to air and their opercular movement did not cease for close to 5 h (293 min) (Rahmanifarah et al., 2011).

3.4 Nile tilapia (*Oreochromis niloticus*)

4 199 567 tonnes of Nile tilapia were produced in 2016. The ability to tolerate a wide range of temperatures, 8°C to 42°C, short time to sexual maturation, and the potential to spawn every 30 days makes the Nile tilapia a highly profitable aquaculture species. This tropical species has a latitudinal range of 32°N – 5°S.

Lambooij et al. (2008) used EEG measurements to thoroughly test different wet electrical stunning parameters with Nile tilapia and concluded that tilapia can be stunned almost instantaneously using top-to-bottom applied current density of 1.0 A_{rms}/dm^2 or using side-to-side applied current using 0.4 A_{rms}/dm^2 at 50 Hz sinusoidal AC for 1 s. A side-to-side 1 s application of pulsed square wave alternating current (density of 0.6 A_{rms}/dm^2 / 133 Hz and 43% duty cycle) was also found to immediately stun tilapia (Lambooij et al., 2008).

A later study using wet electrical stunning (154 V / 50 Hz / 8 A for 3 min) reported similar results with loss of all visual indicators of consciousness 30 s after the current was shut off (Oliveira Filho et al., 2015). After stunning, tilapia had a plasma cortisol concentration of 15 ng/ml (Oliveira Filho et al., 2015), well within the range of the 5-60 ng/ml control cortisol levels found in other studies for unstressed fish (Barreto & Volpato, 2004; Auperin et al., 1997).

The use of stronger electrical currents is also being researched as a potential stunning method for Nile tilapia in open water farms. Mahmoud et al. (2019) evaluated the process of electrofishing of Nile tilapia in a poly cultured fish farm using a generated unit that emitted an electrical current of 220 V AC. Electrical stunning caused immediate loss of equilibrium and tremors in the fish as well as fine blood spots in the skin (Mahmoud et al., 2019). No fish recovered during this process.

Wang et al. (2017) stunned tilapia by submersion in a tank containing freshwater diffused with nitric oxide (NO) until fully saturated. Though the loss of visual indicators of consciousness took 20 min fish did not display any aversive behavior or stress response during stunning. After stunning, Nile tilapia had a plasma cortisol concentration of 303 ± 44 ng/ml, indicating fish did experience stress during the procedure (Wang et al., 2017). The same study measured cortisol levels in Nile tilapia stunned using percussion and reported concentrations of 412 ± 63 ng/ml, indicating fish experienced stress during this process (Wang et al., 2017).

When tilapia were stunned using CO_2 saturated water, unconsciousness was reached after 30 min and fish had an average cortisol of 17 ng/ml (Oliveira Filho et al., 2015). Tilapia in this study did not show any aversive behavior but did spend some time at the surface of the water taking in air (Oliveira Filho et al., 2015).

When tilapia were placed in a tank containing a 1:1 ratio of ice to water it took 20 min for fish to become unconscious in a study by Oliveira Filho et al. (2015). After, fish showed a low cortisol level of 22 ng/ml indicating fish experienced minimal stress during the stunning process (Oliveira Filho et al., 2015).

When fish were asphyxiated in air in a study by Mahmoud et al. (2019) fish showed a strong aversive reaction. For the first 3 min, fish exhibited rapid breathing, gasping and vigorous movement which resulted in lesions and loss of scales. Tilapia ceased movements 30 min after asphyxiation began (Mahmoud et al., 2019).

3.5 Channel catfish (*Ictalurus punctatus*)

The channel catfish is a species of North American freshwater catfishes with a latitudinal range of 55°N - 25°N. In 2016, 432 932 tonnes of channel catfish were produced for aquaculture.

Small (2003) assessed the efficacy of clove oil stunning on channel catfish. 200 ppm clove oil was added directly to an aquarium for 30 min to anesthetize 10 juvenile catfish. Once fish were visually deemed unconscious, 6 fish were collected and had blood drawn for plasma cortisol analysis. The results showed that clove oil did not significantly increase cortisol levels from baseline value, <10 ng/ml, for the duration of treatment (Small, 2003).

Similar results were found in a study that exposed juvenile channel catfish to clove oil concentrations varying between 0 and 100 ppm (Bosworth et al., 2007). When catfish were exposed to 40, 50 and 100 ppm clove oil concentrations over a 60 min period, all catfish lost equilibrium within 3 min of exposure. Lower doses of clove oil appeared to calm the fish and fish did not struggle when being handled, however, equilibrium was maintained and fish remained conscious. Catfish exposed to 100 ppm clove oil did not recover after the test (Bosworth et al., 2007). The same study examined the effects of water temperature on induction time using 35 ppm clove oil on juvenile catfish and found that induction levels decreased as temperature increased (Bosworth et al., 2007).

When market-weight channel catfish were exposed to 30-35 ppm clove oil during rested harvesting plasma cortisol levels were 4-8 times lower than levels after traditional harvesting, 9.2-22.6 ng/ml compared to 82.2 ng/ml, respectively (Bosworth et al., 2007).

In an early study by Boggess et al. (1973) catfish stunned using AC or DC current (400 V) experienced convulsions post-stun. Submersion in CO_2 saturated water resulted in loss of visual indicators of consciousness after 3 min. When catfish were eviscerated and decapitated after 3 h of being asphyxiated on ice (1.7°C) it was found that the gall bladders were enlarged and in danger of being ruptured during decapitation (Boggess et al., 1973).

3.6 African sharptooth catfish (*Clarias gariepinus*)

The African sharptooth catfish is a subtropical species of freshwater catfish, with a latitudinal range of 42°N – 28°S. In 2016, just over 231 090 tonnes of African sharptooth catfish were farmed for aquaculture.

22 catfish surgically implanted with EEG electrodes in order to analyze brain activity were stunned using a 16 mm cone-shaped needle that compressed air in 3 directions using a pressure of 8

bars and an injection pressure of 3 bars over 1.5 s (Lambooij et al., 2003). After captive needle stunning the EEG recording indicated that fish were unconscious as a result of the air injection causing lacerations in the brain. Fish were unable to swim normally and experienced severe but temporary convulsions with movement ceasing after 38 ± 50 s. However, when placed in a recovery tank some stunned catfish displayed uncoordinated swimming movements but were unable to maintain equilibrium (Lambooij et al., 2003). This is most likely because the spinal cord, which is not destroyed during captive needle stunning, controls much of coordinated movements in catfish (Lambooij et al., 2003).

A study by Lambooij et al. (2004) assessed the effects of dry head-only electrical stunning on EEG equipped African sharptooth catfish. An electrical current of 362 ± 32 V / 629 ± 180 mA applied for 1 s successfully stunned 31 restrained catfish for 23 ± 8 s according to EEG recordings (Lambooij et al., 2004). The same research group later found that catfish could be successfully stunned for 28 ± 8 s using wet electrical stunning with a current of 291 ± 5 V / 1.60 ± 0.11 A applied for 1 s (Lambooij et al., 2006). Given the short time of unconsciousness seen with both dry, head-only stunning and wet stunning, it is important that catfish be killed as soon as possible after stunning to prevent possible recovery.

When submerged in ice water (0.1 ± 0.5°C) the EEG of African sharptooth catfish showed brain activity transitioned from a conscious to an unconscious state roughly 5 min after submersion, indicating that fish were unconscious, and low brain activity was seen after 10 min (Lambooij et al., 2006). Responses to needle scratches applied to the tail disappeared between 5 and 20 min after immersion, indicating that some catfish were not responding to stimuli while conscious and others were responsive when unconscious. Measurements of heart rate using ECG recordings showed that catfish experienced a prolonged period of increased heart rate after submersion, which may indicate that ice chilling is a stressful method of inducing unconsciousness (Lambooij et al., 2006).

African sharptooth catfish responded to noxious stimuli for 15 min after having gills cut with no pre-slaughter stunning (Lambooij et al., 2004). A visible response to noxious stimuli was also seen in catfish with gills cut after undergoing head only electrical stunning (Lambooij et al., 2004). The EEG recording showed that these fish had no brain activity 12 ± 5 s after gill cutting, but 2 fish showed a visible response to noxious stimuli after 2 and 5 min (Lambooij et al., 2004).

3.7 European seabass (*Dicentrarchus labrax*)

191 003 tonnes of European seabass were harvested for aquaculture in 2016. This popular species of seabass has a latitudinal range of 72°N - 11°N.

When submerged in an ice slurry (1 ± 1°C) comprised of a 1:2 ratio of flake ice to sea water, European seabass swam quickly or normally for the first 2 min of treatment before slowing and equilibrium and slowing of ventilation (Zampacavallo et al., 2015). After 9 min all fish ceased movement but some were still responsive to external stimuli. All seabass were visually deemed as completely unconscious or dead 23-30 min after the beginning of treatment (Zampacavallo et al., 2015). Similar times to visually deemed unconsciousness or death were seen by Bagni et al. (2007) and Acerete et al. (2009), taking 20 min and 34 min, respectively, in seabass submerged in an ice slurry. Bagni et al. (2007) found that when seabass are chilled in a crowded environment time to unconsciousness was significantly longer, increasing from 20 min (uncrowded) to 45 min (crowded). When plasma cortisol levels were assessed, Acerete et al. (2009) saw a 5-fold increase in concentration from unstressed cortisol levels of 60 ng/ml when bass were killed in ice slurry.

When seabass were live chilled in an ice slurry with dissolved gas (70% N_2 + 30% CO_2), the process appeared to be more stressful than ice slurry alone (Zampacavallo et al., 2015). After immersion, fish demonstrated aversive behavior for the first 3 min of submersion, followed closely by loss of equilibrium. After 6-11 min in treatment fish ceased moving but some still reacted to stimulus. Death occurred 14-19 min after the start of treatment. Time to death was shortened to 10 min when the dissolved gas mixture was 40% N_2 + 60% CO_2 (Zampacavallo et al., 2015). When using wet electrical stunning by exposure to either single phase (50 Hz / 40 V for 4 min) or dual phase electrical current (400 Hz / 120 V for 1 min followed by 50 Hz / 40 V for 3 min) European seabass immediately lost visual indicators of consciousness and did not react to needle scratches applied near the lateral line once the current was turned off (Zampacavallo et al., 2015).

When seabass were asphyxiated in air they showed an 8-fold increase in plasma cortisol levels compared to control values in a study by Acerete et al. (2009) and time until death was 34 min after the start of treatment. A similar time to death was seen by Bagni et al. (2007), with uncrowded bass dying after 35 min in air. Crowded bass took significantly longer to die, with movement and visual indicators of consciousness ceasing after 70 min (Bagni et al., 2007).

Submersion of European seabass in CO_2 saturated water produced a 5-fold increase in cortisol levels compared to baseline values with death occurring 16 min after the beginning of treatment (Acerete et al., 2009).

When European seabass were submerged in 15°C water mixed with 30 mg/L clove oil visual indicators of consciousness were lost within 2 min with fish appearing calm during induction (Mylonas et al., 2005). Recovery took slightly longer, with fish requiring 4 min to resume normal swimming (Mylonas et al., 2005). Temperature of the water was significant with regards to induction time, with fish held at lower temperatures taking longer to lose visual indicators of consciousness as well as having longer recovery times (Mylonas et al., 2005).

3.8 Gilthead seabream (*Sparus aurata*)

In 2016, 185 980 tonnes of gilthead seabream were produced for aquaculture. The seabream is a subtropical species with a range of 62°N - 15°N.

Ice chilling is the industry standard for processing of gilthead seabream (Huidobro et al., 2000). After immersion in a 3:1 ratio of ice to seawater (0.8 ± 0.2°C), gilthead seabream lost both visual indicators of consciousness and brain activity (VERs) after 5 min according to EEG recordings (Van de Vis et al., 2003). Seabream also showed visual indicators of stress, including vigorous movements, with this method (Van de Vis et al., 2003). In a study by Bagni et al. (2007), seabream submerged in an ice slurry (1.4°C) took an average 20 min to lose consciousness when uncrowded and 40 min when crowded. Extreme struggling, indicating stress, was noted in both cases (Bagni et al., 2007).

Bagni et al. (2007) asphyxiated seabream in air under both crowded and uncrowded environments. Fish asphyxiated when uncrowded struggled for 25 min in air, while crowded fish struggled for 50 min. Van de Vis et al. (2003) also noted escape attempts in seabream when asphyxiated in air though the average time to loss of consciousness indicators was much shorter, taking 4 min for visual indicators to be lost and 5.5 min for VERs to be lost.

When stunned using a pneumatic gun operating at 6 bar, gilthead seabream lost visual indicators of unconsciousness and VERs instantly with no recovery (Van de Vis et al., 2003).

When stunned for 1 s using a head-only application of electrical current (50 Hz / 80 V / 27-200 mA AC), only 1 seabream of 10 showed signs of unconsciousness and recovered VERs after 37

s (Van de Vis et al., 2003). When stunned for 10 s using a slightly higher electrical current (50 Hz / 80 V / 400+ mA), 9 of the 10 seabream tested were immediately stunned. Of the 9 stunned fish, VERs were recovered in 3 fish within 16 s of current stopping with the no recovery after 10 min in the other 6 fish (Van de Vis et al., 2003). While the minimum current required to achieve the desired outcome is not yet known, at least 200mA is required to stun seabream during head-only electrical stunning according to Van de Vis et al. (2003).

When gilthead seabream were transferred to a tank containing 15°C water mixed with 55 mg/L clove oil it took less than 3 min for fish to lose visual indicators of consciousness (Mylonas et al., 2005). During induction, seabream temporarily made swimming attempts after losing equilibrium. When recovering, seabream made uncoordinated swimming attempts as early as 40 s before equilibrium returned. Visual indicators of consciousness were apparent within 4 min of transfer to a recovery tank (Mylonas et al., 2005).

3.9 Barramundi (*Lates calcarifer*)

The barramundi, also called Asian seabass, is a tropical fish with a range of 49°N - 26°S and are the most important commercial fish in Australia. In 2016, 56 933 tonnes of barramundi were produced for aquaculture.

A study by Wilkinson et al. (2008) found that the process of rested harvest, adding a stock solution of the clove oil derivate AQUI-S (25 mg/L) before transferring fish to an ice slurry for 15 min, resulted in low cortisol levels (1.03 ± 0.44 ng/ml). A similar study by Wilkinson (2012) found that even though the cortisol levels were considered low in both groups of barramundi anesthetized with AQUI-S before transfer to ice slurry had a significantly lower cortisol level than fish that were moved directly to ice slurry.

When exposed to simulated conventional harvest techniques, barramundi had an average cortisol level of 42.99 ± 12.00 ng/ml (Wilkinson et al., 2008). Conventional harvest techniques consisted of rapid removal of water followed by air exposure for 3 min. After 3 min in air, water was added back and fish were netted and transferred to ice slurry for 15 min. 9 h post-harvest, there was still a significant increase in plasma cortisol levels, 25.55 ± 1.30 ng/ml, indicating that conventional harvest techniques are a significant source of stress for barramundi. During a later experiment, Wilkinson (2012) noted that simulated harvest technique prompted escape responses in barramundi when netted.

3.10 Cobia (*Rachycentron canadum*)

43 107 tonnes of cobia were farmed for aquaculture in 2016, with the highest capture rates occurring in the Philippines and Pakistan. The cobia has a wide range of 47°N - 37°S and is found mostly worldwide in subtropical and tropical waters, with the exception being the eastern Pacific and Pacific plate.

Trushenski et al. (2012) examined the response of juvenile cobia to CO_2 stunning (736 ± 21 mg/L, 27 °C water) and found that fish experienced color changes on the skin and were hyperactive. It took 2.7 ± 0.1 min for fish to be visually deemed unconscious, and once fish were moved to a recovery tank a response to tactile stimulus was seen 1 min later. Equilibrium was recovered after 3.2 ± 0.1 min. Fish in this treatment experienced a significant increase in cortisol, 450 ng/ml compared to resting levels of 40 ng/ml (Trushenski et al., 2012). Similar results were found with cobia stunned using CO_2 saturated water (pH of 4.5, 23.4°C water) in a study by Baldi et al. (2018), with fish making

violent escape attempts and displaying aversive behavior during the first 31 min of treatment. Fish were deemed unconscious through visual cues after 48 min (Baldi et al., 2018).

Trushenski et al. (2012) used electrical stunning on juvenile cobia and reported that a pulsed direct current (100 V / 30 Hz / 25% duty cycle for 5 s) induced visually determined unconsciousness after 0.2 ± 0.1 s. Recovery of equilibrium and response to tactile stimulus were seen after 0.6 ± 0.1 min and 0.8 ± 0.1 min, respectively (Trushenski et al., 2012). Electrically stunned cobia showed body tensing during treatment, combined with gasping and fin extension, but all responses ceased after exposure ended. Cortisol levels increased to 375 ng/ml 30 min after exposure but reduced to near resting value 1-hour after treatment, 75 ng/ml compared to 25 ng/ml resting (Trushenski et al., 2012). Baldi et al. (2018) found similar results with electrical stunning of 6-month old cobia (1.68 kg ± 0.47 kg) with 150 V / 60 Hz / 7.3 A for 3 min. Fish lost visual indicators of consciousness almost instantly once current began (Baldi et al., 2018).

When submerged in a 1:1 ratio of ice:water, cobia made escape attempts and displayed aversive behavior for 7 min. After, fish moved little but showed increased production of mucus and attempted to breath at the surface of the ice slurry. After 17.5 min, all visual indicators of consciousness were gone (Baldi et al., 2018).

3.11 Meagre (*Argyrosomus regius*)

In 2016, 23,439 tonnes of meagre were harvested for aquaculture according to the FAO. This subtropical fish has a range of 65°N - 6°S.

Cárdenas et al. (2016) transferred juvenile meagre to tanks containing seawater (18°C) mixed with clove oil in concentrations of 30, 40 or 50 mg/L to determine the efficacy of the anesthetic (Cárdenas et al., 2016). While meagre anesthetized with 40 or 50 mg/L lost visual indicators of consciousness in less than 3 min, fish anesthetized with 30 mg/ml did not lose all visual indicators after 15 min of submersion. Recovery times for all three concentrations was less than 5 min (Cárdenas et al., 2016). When induced using a low anesthetic concentration, 1 mg/L, plasma cortisol levels increased to twice the concentration of unanesthetized meagre, ~40 ng/ml compared to ~20 ng/ml, respectively (Cárdenas et al., 2016).

A separate study using juvenile meagre measured induction times using clove oil at concentrations of 25, 40, 55, 70 and 85 mg/L in seawater (18°C) (Barata et al., 2016). Meagre lost visual indicators of consciousness within 10 min of submersion in seawater containing 40 mg/L or higher concentrations of clove oil (Barata et al., 2016). When fish anesthetized with 85 mg/L clove oil had their skin pricked using a needle they were not responsive. Meagre induced using a lower anesthetic concentration, 10 mg/L, had plasma cortisol values significantly higher than that of unanesthetized fish, 140 ± 129 ng/ml compared to 86 ng/ml (Barata et al., 2016). A study by Fanouraki et al. (2011) reported plasma cortisol concentrations of <10 ng/ml for unstressed meagre.

3.12 Pacu (*Piaractus mesopotamicus*)

The pacu is a subtropical species found in the range of 15°S - 38°S. In 2016, the FAO reported 15,847 tonnes of pacu were harvested for aquaculture.

In a study by Rucinque et al. (2018) pacu were divided into two groups and wet electrically stunned using a backpack electrofisher with either 205 V at 50 Hz, duty cycle of 70% and 1.3 A for 45 s or with 400 V at 30 Hz, duty cycle of 30% and 0.9 A for 30 s. In both groups, fish immediately lost all visual indicators of consciousness and did not respond to needle scratches. Pacu stunned with

205 V remained unconscious for 61.7 ± 13.4 s while those stunned with 400 V were unconscious for 50.1 ± 9.6 s (Rucinque et al., 2018).

Similar results were found by Oliveira Filho et al. (2016) when pacu were wet electrically stunned with 100, 150 or 200 V for 180 s using either AC or DC current. Regardless of current type used, all pacu exhibited no visual signs of stress and were visually deemed as unconscious after current ceased. Following exposure to 200 V AC, pacu were visually deemed as unconscious for up to 4 min after the stun with minimal internal bleeding upon later inspection (Oliveira Filho et al., 2016).

3.13 European eel (*Anguilla anguilla*)

In 2016, European eels accounted for 6 994 tonnes of aquaculture production. The European eel has a range of 75°N - 8°N, and while considered a temperate species these eels are capable of thriving in temperatures between 0°C and 30°C.

Two different studies showed that >90% of eels were rendered unconscious (determined by seizure activity on the EEG) both individually, when either dry head-only electric shock was used at 255 ± 4 V / 50 Hz / 545 ± 32 mA AC for 1 s (Lambooij et al., 2002c), or in batches of eels exposed to 0.636 ± 0.040 A/dm^2 / ~200 V during wet electrical stunning for ~2 s (Lambooij et al., 2002d). In a study by Robb et al. (2002), dry head-only application of a current of 65 V and 50 Hz for 1 s consistently produced seizure activity, indicating unconsciousness, in eels. When this current was extended to 30 s it was possible to kill eels (Robb et al., 2002).

Electrical stunning while submerged in water with dissolved nitrogen has produced conflicting results. When eel were stunned using an electrical current of 196 ± 2 V / 0.693 ± 0.011 A/dm^2 for 1 s followed by 50 V / 0.165 A/dm^2 for 5 min in water flushed with nitrogen no brain activity was seen post-stun. However, 1 eel was able to right itself after 60 min while non-responsive to needle scratch (Lambooij et al., 2002d). In a different study, eel were observed moving and breathing after being placed in a tank of freshwater that had oxygen saturation reduced to 20% by bubbling nitrogen gas in and exposed to an electrical current of 250 V (Hz unspecified) for 10 s followed by 80 V (Hz unspecified) for 7 min (Morzel & Van de Vis, 2003).

Morzel and Van de Vis (2003) also placed eels in a dry tank with salt equivalent to 1/10 the weight of the eels, after which eels were rinsed and gutted. Eels showed strong aversive reactions and escape attempts in the salt bath and movement ceased after 15 min, most likely from exhaustion. When filleted, eel slaughtered in the salt bath had a number of broken bones, more than what was seen with electrical stunning, likely due to increased physical movement (Morzel & Van de Vis, 2003).

Verheijen and Flight (1997) placed eels in a dry salt bath for 20-30 min, after which water was added. During the time in the dry salt, eels showed "violent and well-coordinated escape movements" (Verheijen & Flight, 1997). Eventually, movement intensity decreased and in some cases ceased altogether. However, once water was added some eels began moving again. After 20-30 min in the brine almost all eels were unmoving, which is considered dead under commercial conditions (Verheijen & Flight, 1997). Once moved to a tank containing tap water, all 14 eels began moving again and were noted to be in poor health. "The eyes were turbid. Loosened mucus and epidermis rags formed a whitish wraparound." All eels died within 18 h after transfer to the tap water tank (Verheijen & Flight, 1997).

Captive needle stunning with a shooting pressure of 8 bar and an air injection of 3 bar over the course of 1.5 s was enough to render most eels in the 700-800 g range unconscious, as determined

by seizure activity on EEG recordings (Lambooij et al., 2002a). Captive needle pistol immediately induced low or no brain activity during EEG recording, however, a few eels had muscle cramps that lasted over an hour (Lambooij et al., 2002a).

When submerged in an ice slurry eels initially showed aversive behaviors (Lambooij et al., 2002b). However, after 12 ± 5 min, the time it took for body temp to lower to 8°C, all responses to pain stimuli disappeared (Lambooij et al., 2002b). Following the time in ice slurry the eels were transferred to a cold brine at -18°C, during which responses to stimuli remained in 3/18 eels. EEG in brine indicated that some brain activity was still occurring at the time of transfer. No eels recovered after a stay of 15 min in cold brine (Lambooij et al., 2002b).

Verheijen and Flight (1997) decapitated 9 eels just after the pectoral fin using a razor blade. 6 heads were kept and observed in a bowl of 20-22°C water and 3 heads were kept in air. The heads kept in water exhibited respiratory movements and opening of the mouth for 25-55 min after submersion. Heads kept in air stayed alive for 1.25 h, 6 h, and 7.75 h (Verheijen & Flight, 1997). Signs of life included respiratory movements, opening of the mouth, twisting movement and movement of the pectoral fins. The long signs of life after decapitation indicate that eels were still sensible during this time (Verheijen & Flight, 1997).

3.14 Senegal sole (*Solea senegalensis*)

The Senegal sole is a tropical flatfish with a range of 47°N - 16°S. 1 510 tonnes of Senegal sole were farmed for aquaculture in 2016.

Ribas et al. (2007) compared the cortisol response of Senegal sole during different stunning methods to levels of unstressed sole (~1 ng/ml). One method was hypothermia, transferring fish from 16°C water to 2-4°C water, which resulted in a plasma cortisol concentration 35 times higher than unstressed levels. Another method was submersion in a 1 ml/L clove oil in water solution, which resulted in a similar cortisol concentration as unstressed fish. The last method was asphyxia on ice, resulting in the highest cortisol level of all methods, 263 times higher than unstressed levels (Ribas et al., 2007).

When Senegal sole were exposed to a much higher concentration of clove oil, 80 mg/L, in 14°C water for 30 min, plasma cortisol levels increased 300% compared to unstressed sole. However, after 24 h cortisol levels returned to unstressed levels (Weber et al., 2009).

3.15 Spotted snakehead (*Channa punctata*)

The spotted snakehead is a tropical fish belonging to the perch family. In 2016, 584 tonnes of spotted snakehead were produced for aquaculture. The estimated latitudinal range is between 30°N - 0°N.

Kumari et al. (2018) exposed spotted snakehead to three different concentrations of clove oil, 50, 100 and 200 µl/L, with 5 fish used for each concentration. The maximum dose of clove oil had the shortest induction time, however appears to be toxic to fish at that concentration. "Causes mucus exudation, partial lamellar fusion in the gills and dermal lifting of buccal epithelium, all indicators of acute stressful conditions of the fish (Kumari et al., 2018)." For this reason, a concentration of 100 µl/L was recommended for anesthesia induction of snakehead as it did not appear to cause any adverse reactions (Kumari et al., 2018).

3.16 Common/Dover sole (*Solea solea*)

The common sole is a flatfish with a latitudinal distribution range of 67°N - 17°N. 11 tonnes of sole were farmed for aquaculture in 2016, but 32 057 tonnes were captured globally.

In a study by Daskalova et al. (2016a) 33 common sole were stunned using tail-first dry electrical stunning followed by ice chilling. The sole were divided into two groups, short and long stun. The short stun group (n=10) received a 1 s stun of 1.22 ± 0.68 A_{rms} and was assessed for immediate unconsciousness determined by EEG measurements at 30 s and at each min following for 5 min. After the stunning process, 1 sole did not show a clear epileptiform insult indicating it was still conscious, and 1 sole showed EEG indicators of unconsciousness 30 s and 1 min after testing but not during stunning (Daskalova et al., 2016a). The remaining 8 sole showed no reactions to a needle scratch 1 min after stunning but EEG recordings indicated that fish regained consciousness before the min was over (Daskalova et al., 2016a). Following the long stun (20 s stun comprised of a 1 s stun of 1.20 ± 0.59 A_{rms} followed by a 19 s maintenance stun of 0.36 ± 1.26 A_{rms}) and ice chilling all sole were rendered unconscious and remained so for 5 min after the stun (Daskalova et al., 2016a). However, "minimal brain activity" was seen in 15 sole during the 5 min following, with 1 sole responding to needle scratch at min 1 and 4 and 2 sole responding to scratches at min 3 and 5. None of the sole responded to needle scratches 10 min after the stun and remained unresponsive until the end of testing at 75 min (Daskalova et al., 2016a). When fish were physically tapped, 2 sole responded 2 min after stun, 9 responded at 3 min, 12 at 4 min and 13 at 5 min (Daskalova et al., 2016a). A study by Llonch et al. (2012) assessed the effects of dry electrical stunning using 0.65 ± 0.23 A_{rms} / 100 Hz for 1 s on common sole. Using this current 22 out of 25 sole were rendered unconscious according to EEG recordings. When the duration of exposure was extended to 5 s followed by ice chilling, all sole were instantly rendered unconscious with no fish responding to noxious stimuli (Llonch et al., 2012). Only 1 out of 10 sole showed EEG indicators of consciousness at 2 and 3 min after stunning (Llonch et al., 2012).

3.17 Lebranche mullet (*Mugil liza*)

4.38 tonnes of Lebranche mullet were farmed in aquaculture in 2016. This subtropical fish has a wide range of 32°N - 51°S.

Juvenile Lebranche mullet were individually anesthetized using eugenol, a clove oil extraction, in concentrations of 50, 70, 90, and 110 mg/L mixed in 23.8 ± 0.5ºC water (da Silva Braz et al., 2017). Induction times were found to decrease with higher concentrations and visual indicators of consciousness were lost within 210 s of submersion for all concentrations. 70 mg/L eugenol was found to be most efficient concentration, with induction taking 170.1±16.4 s and full recovery taking 407.7±73.6 s (da Silva Braz et al., 2017).

3.18 Marbled spinefoot (*Siganus rivulatus*)

In 2016, 0.02 tonnes of marbled spinefoot were farmed for aquaculture purposes. This perch-like fish has a range between 32°N - 36°S.

Ghanawi et al. (2011) found that 70 mg/L clove oil was the most effective dose on juvenile marbled spinefoot. When spinefoot were transferred to 23.02 ± 1.08°C water mixed with 70 mg/L clove oil visual indicators of consciousness were lost 1 min after submersion. Recovery time for these fish was slightly less than 5 min (Ghanawi et al., 2011). A later study by Santos et al. (2015)

investigated the effects of water temperature on the efficacy of 70 mg/L clove oil on marbled spinefoot. Time to loss of visual indicators of consciousness decreased as temperature increased, with the difference between 20ºC and 30ºC water nearly halving the induction time from 1.43 min to 0.86 min (Santos et al., 2015). Time to recovery was also heavily dependent on temperature, with spinefoot recovering in 3.68 min at 30ºC and 5.31 min at 20ºC (Santos et al., 2015).

4 Discussion

While knowledge on stunning and killing methods during slaughter and their impacts on fish welfare has increased in recent years, it is apparent that there are still knowledge gaps that need to be filled. In 2016 there were over 400 reported species farmed in aquaculture and of these 167 are defined as tropical or subtropical species (FAO, 2018c). Of these tropical and subtropical fishes we found no information available with regards to fish welfare during stunning and killing on 149 species. There were, however, a vast number of papers available that examined the effects stunning, slaughter and storage had on fillet quality.

It is well known that improper stunning techniques can lead to blood spots, muscle hemorrhages, and metabolic changes that affect the taste and texture of fillets (Barton 2002; Poli et al. 2005). Unfortunately, it is not always the procedures that result in the highest quality fillets that are also the most humane. Most stunning, killing and slaughter methods are chosen because they are easy to administer and do not have any negative effects on the products rather than a quick and effective method that leave the animal unconscious until death (Ross et al., 2009). With so few studies available that assess the welfare of farmed fish it is very possible that less than ideal practices continue to be used due to a lack of information, as opposed to a lack of regard for welfare.

Another limitation in the available literature is the difficulty to accurately assess consciousness, or lack thereof. Changes in mental state are not always easily discernable through visual assessment alone as animals can both show behavioral signs of consciousness after the brain activity is lost and, perhaps more concerning, an animal can become paralyzed and incapable of responding to its surrounding while neither insensible nor unconscious (Van de Vis et al., 2003). In the literature there are several examples of such mismatches between visual indicators of consciousness and consciousness determined by measurements of brain activity (i.e. EEG). African sharptooth catfish were reported swimming in an uncoordinated manner even after an effective captive needle stun (Lambooij et al., 2003) and common sole that were unconscious according to EEG still responded to needle scratches after being electrically stunned (Daskalova et al., 2016b). The opposite problem was reported for both common sole stunned using electricity (Daskalova et al., 2016b) and African sharptooth catfish submerged in ice slurry (Lambooij et al., 2006) with fish being non-responsive to needle scratches but conscious according to EEG recordings. This highlights not only the need for additional research with regards to brain activity in fish, but also the importance of proper evaluations of the methods used to do so. Measurements of EEG in a living vertebrate is essentially the measurement of “chaos” in the brain and a single stimulus (i.e. a needle scratch) can easily be lost in the noise. This is why visually evoked responses (VERs) are usually assessed using the averaged consecutive summation of light stimulus, usually no less than 100 light flashes (Kestin et al., 1991; Robb et al., 2000; Retter et al., 2018). This results in a clearer signal, with variable noise getting averaged out while the consistent VERs stay visible in the recording. Moreover, for many animals, including fish, the detection of potentially painful stimuli is usually accompanied by a reflex withdrawal response (Sneddon et al., 2015). As such reflexes are often mediated by a simple reflex

arc and do not require any input from the brain researchers need to be cautious when using a potentially painful stimulus to assess consciousness as a reflex can also occur in an unconsciousness animal.

Reliable measurements of consciousness are also important when investigating the recovery time of various stunning methods, as was shown in common carp that brain activity can return long before any visual indicators of consciousness during electrical stunning (Retter et al., 2018). This is especially important when considering stunning methods that may cause immobilization without unconsciousness, as was seen in electrical stunning (Daskalova et al., 2016a; Readman, 2015, Van de Vis et al., 2003) or submersion in ice slurry (Robb & Kestin, 2002). Unfortunately, there are very few papers analyzing brain activity and the prevalence of different brain waves in fish and if those waves are consistent throughout species.

4.1 Accuracy in methodology

There is a huge need for accuracy with regards to method descriptions, though this is not limited to research involving tropical and subtropical fish. Reporting the specifics in a paper is crucial for both understanding the topic as well as repeatability, and validity, of an experiment. How electrical stunning parameters should be described depends on whether the current is delivered dry or wet and in fresh or saltwater. Wet electrical stunning should also be accompanied with information on water conductivity and whether the current delivered was alternating or direct, as that affects not only how the electrical current is applied but also how the fish are affected. Additionally, because the solubility of water is temperature dependent, it is crucial that temperature be listed when using carbon dioxide or anesthesia as a stunning method. The pH of the water is also important to mention with carbon dioxide stunning, as a pH higher than 5 may indicate that the water is not fully saturated (Anonymous, 1995) and fish may take longer to lose visual indicators of consciousness, if indicators are lost at all.

Additional research should be conducted in cases where different studies use the same method and report wildly different results. For example, when using immersion in carbon dioxide saturated water to stun juvenile cobia, Trushenski et al. (2012) reported that visual indicators of consciousness were lost 2.7 min after immersion. However, Baldi et al. (2018) reported that visual indicators took 48 min to be lost using the same method. While there was a 4ºC difference in water temperature between the two studies that would not be enough to account for such a difference in induction times. Conflicting results were also reported with gilthead seabream asphyxiated in air. In an early study by Van de Vis et al. (2003), gilthead seabream were reported to lose visual indicators of consciousness after 4-5 min of asphyxia in air. A later study by Bagni et al. (2017) reported seabream took between 25 and 50 min depending on fish density to lose visual indicators of consciousness during air asphyxiation. Conflicting results between studies could be due to any number of factors. However, when developing best practice stunning and killing methods it is important that procedures be based off consistent results rather than results that are convenient or appealing but lacking scientific support from other studies.

4.2 Asphyxia, decapitation and exsanguination

Asphyxia in air is possibly the easiest and cheapest of all stunning methods but is not efficient, with fish sometimes taking hours to lose visual indicators of consciousness. Common carp were reported to show aversive behavior when asphyxiated in air and took nearly 5 h to lose visual indicators of consciousness (Rahmanifarah et al., 2011). A separate study reported common carp experienced a

very significant increase in cortisol levels (633-800+ ng/ml) after asphyxiation in air for 90 min (Daskalova et al., 2016b). Nile tilapia took less time than carp to lose indicators, 30 min, but also exhibited stressed behaviors (Mahmoud et al., 2019).

Time to loss of visual indicators also appears to be affected by fish density. Bagni et al. (2017) reported that European seabass asphyxiated in air took twice as long to lose visual indicators of consciousness when crowded compared to uncrowded, 70 min and 35 min, respectively. In a separate study, European seabass lost visual indicators after 34 min of asphyxiation in air and experienced an 8-fold increase (480 ng/ml) in cortisol levels compared to unstressed fish (60 ng/ml) (Acerete et al., 2009). Gilthead seabream were also found to asphyxiate slower when crowded, taking 50 min to lose visual indicators of consciousness compared to 25 min when uncrowded (Bagni et al., 2017). However, an earlier study assessed gilthead seabream asphyxiated in air using EEG and found that VERs were lost after 5 min, 1 min after visual indicators were lost (Van de Vis et al., 2003).

Asphyxiation on ice is also a stressful and time consuming stunning method. After 3 h of being covered with ice, channel catfish had enlarged gall bladders that made fillet harvesting nearly impossible without bursting the organ (Boggess et al., 1973). Senegal sole were reported to have a cortisol level drastically higher than unstressed fish after asphyxiation on ice, 263 ng/ml compared to 1 ng/ml (Ribas et al., 2007). The long time required for fish to asphyxiate in air or on ice, as well as the increased cortisol levels and aversive behavior reported point to this method being extremely stressful to some fish and is not recommended.

Decapitation was only reported for 1 species, European eels. When European eels were decapitated and their heads submerged in water they continued to make respiratory movements for nearly an hour after immersion. Decapitated eel heads left exposed to air exhibited respiratory, twisting, and pectoral fin movements for up to 8 h (Verheijen & Flight, 1997).

4.3 Carbon dioxide and Nitric oxide

CO_2 stunning is inexpensive and easy to implement on large groups of fish, but can cause aversive behavior in some species. When common carp and cobia were submerged in water fully saturated with carbon dioxide, they made numerous escape attempts and exhibited aversive behavior until visual indicators were lost (Rahmanifarah et al., 2011; Baldi et al., 2018). However, Nile tilapia appear to experience little stress with carbon dioxide stunning, with no aversive behavior and a cortisol level equal to that of unstressed fish (Oliveira Filho et al., 2015).

Cortisol levels and the time necessary for visual indicators to be lost with carbon dioxide stunning varies greatly depending on which species is being stunned. Channel catfish (Boggess et al., 1973) and one study for cobia (Trushenski et al., 2012) reported time to loss of visual indicators as 3 and 2.7 min, respectively. Trushenski et al. (2012) reported cobia experienced a cortisol level 11 times higher post-stun than that of unstressed cobia when stunned using carbon dioxide. European seabass lost visual indicators after 16 min of submersion and experienced a nearly 6-fold increase in cortisol levels compared to unstressed fish (Acerete et al., 2009). Common carp were also reported to have a significantly increased cortisol level after stunning (Varga et al., 2014) but had a slightly faster induction of 10 min (Rahmanifarah et al., 2011). Nile tilapia required 30 min of submersion before loss of visual indicators but showed no aversive behavior (Oliveira Filho et al., 2015), and Baldi et al. (2018) reported visual indicators of consciousness in cobia up to 48 min after immersion. With regards to fish welfare, carbon dioxide appears to have a negative impact on fish welfare when used with some species. The long induction time in combination with the aversive behavior and increases

in cortisol level point to CO_2 stunning as a poor method for common carp, channel catfish, European seabass and cobia.

Nitric oxide appears to have potential as a stunning method for Nile tilapia. Fish lost visual indicators of consciousness after 20 min of submersion and exhibited a 5-fold increase in cortisol compared to unstressed fish (Wang et al., 2017). Despite the increase in cortisol, tilapia showed no aversive behavior during the stunning process (Wang et al., 2017). Additional research should be conducted to test the potential of nitric oxide as a stunning method for fish and what, if any, the risks are for humans consuming fish stunned using this method.

4.4 Live chilling

Ice chilling is easy and cheap but often causes aversive behavior and with some fish require long immersion times. While there are some conflicting results on immersion times, most studies reported fish requiring 15 min or longer in order to lose visual indicators (Zhang et al., 2017; Zampacavalo et al., 2015; Bagni et al., 2007; Acerete et al., 2009; Baldi et al., 2018).

Similar to asphyxiation in air, time to loss of visual indicators of consciousness when submerged in ice slurry appears to be affected by fish density. Both gilthead seabream and European seabass lost visual indicators after 20 min when live chilled while uncrowded, compared to 40 and 45 min when crowded (Bagni et al., 2007). When European seabass were immersed in an ice slurry with dissolved gas the necessary immersion time decreased compared to other studies (Zampacavalo et al., 2015), but still required upwards of 10 min to be effective. Some fish, such as grass carp (Scherer et al., 2005), silver carp (Zhang et al., 2017), common carp (Rahmanifarah et al., 2011), gilthead seabream (Bagni et al., 2007) and cobia exhibited aversive behavior while submerged in ice slurry. When cortisol levels were measured, Senegal sole (Ribas et al., 2007), European seabass (Zampacavalo et al., 2015), and common carp (Varga et al., 2014) all exhibited significant increases in plasma cortisol concentrations compared to unstressed levels. Surprisingly, Nile tilapia showed cortisol levels equal to that of unstressed fish (Oliveira Filho et al., 2015). Live chilling also runs the risk of immobilization without causing unconsciousness, as was seen with African sharptooth catfish that were non-responsive to stimuli but still conscious according to EEG (Lambooij et al., 2006).

While easy and inexpensive to administer, live chilling appears to be a stressful and aversive method of stunning for some tropical and subtropical species reviewed.

4.5 Percussion and spiking

Manual percussion appears to be a relatively low stress stunning method for silver carp (Zhang et al., 2017), common carp (Retter et al., 2018), and Nile tilapia (Wang et al., 2017) based on reported results, with fish losing visual indicators of consciousness immediately after a successful strike. African sharptooth catfish (Lambooij et al., 2003) and European eel (Lambooij et al., 2002a) were successfully stunned using a 16mm captive needle delivered with 3 bars of pressure and experienced slow muscle cramps after the stun. Gilthead seabream were successfully stunned using a pneumatic gun delivered at 6 bars of pressure (Van de Vis et al., 2003), and common carp were killed using a 60 g captive bolt delivered with 7.5 bars of pressure (Lambooij et al., 2007). Manual percussion and spiking are time consuming, requiring that fish be stunned individually by someone trained to hit a small target. Automated percussion requires specific parameters be met for an effective hit but has the benefit of being able to stun large groups of fish in quick succession. Overall, percussive stunning appears to be an effective low stress stunning method.

4.6 Electrical

When properly administered, electrical stunning causes an immediate loss of visual indicators of consciousness with little to no aversive behavior. Based on studies reviewed for this report, wet electrical stunning appears to be a humane stunning method for grass carp (Scherer et al., 2005), Nile tilapia (Lambooij et al., 2008), African sharptooth catfish (Lambooij et al., 2006), European seabass (Zampacavallo et al., 2015), pacu (Rucinque et al., 2018; Oliveira Filho et al., 2015), and European eel (Lambooij et al., 2002d). Dry electrical stunning was suitable for Nile tilapia (Lambooij et al., 2008), African sharptooth catfish (Lambooij et al., 2004), gilthead seabream when applied at higher amperages (Van de Vis et al., 2003), European eel (Lambooij et al., 2002c), and common sole (Llonch et al., 2012). Electrical stunning in combination with submersion in nitrogen saturated water appears to be particularly effective for European eel (Lambooij et al, 2002d; Morzel & Van de Vis, 2003). A more comprehensive description of electrical stunning parameters used for these species can be found in Table 2.

One downside to electrical stunning is the seemingly sort duration of unconsciousness induced by a successful stun. Most species described in this report had brain activity return within 1 minute of a short stun. Longer stunning durations or increased electrical current could successfully extend the stun duration, such as with dry, head-only stunned gilthead seabream (dry, head-only, 80 V / 50 Hz / 400+ mA AC for 10 s) where unconsciousness lasted 16 s to 10 min after the current was turned off. Longer stuns or stuns followed by chilling in ice water were capable of killing some fish, such as common carp (Lambooij et al., 2007) and common sole (Llonch et al., 2012), with no aversive behavior seen.

Unfortunately, using the incorrect current can cause immobility without unconsciousness, as was reported in common sole (Daskalova et al., 2016a), or bloodspots, seen in electrofished Nile tilapia (Mahamoud et al., 2019). Even worse, an improper stun could not render the fish unconscious long enough to be slaughtered humanely, as was the case for some common carp in field studies by Retter et al. (2018). Electrical stunning may also be stressful for some species of fish, as was seen in the significantly increased cortisol levels of 800+ ng/ml post-stun compared to 5-15 ng/ml in some common carp (Daskalova et al., 2016b) and cobia, 375 ng/ml post stun compared to 25 ng/ml unstressed (Trushenski et al., 2012).

Perhaps the largest downside to electrical stunning is the sheer number of variables that go into a successful stun. Finding the correct combination of voltage, amperage, frequency, conductivity, and more can require a great deal of trial and error. When done successfully electrical stunning appears to be an efficient and humane method of stunning the reviewed species of tropical and subtropical fish.

4.7 Clove oil

Clove oil appears to be an easy and non-aversive method of stunning fish. No fish reviewed in this report displayed aversive behavior after being submerged in water mixed with clove oil at any concentration. Common carp were effectively stunned and lost visual indicators after 3 min of submersion in water with 40-50 ppm clove oil and carp stunned in 100 ppm clove oil did not recover (Bosworth et al., 2007). Senegal sole (Ribas et al., 2007) and channel catfish (Small, 2003) did not experience a significant increase in cortisol level when stunned using 1 ml/L and 200 ppm clove oil, respectively. Barramundi experienced a 42-fold increase in cortisol when exposed to 25 mg/L AQUI-

S, increasing levels from 1 ng/ml to 42 ng/ml (Wilkinson et al., 2008). Other species, such as meagre (Cárdenas et al., 2016; Barata et al., 2016), had plasma cortisol levels slightly higher than those of unstressed fish. Induction time decreased and recovery time increased with increasing dosage when stunning spotted snakehead. At the highest dosage of 200 µl/L clove oil, snakehead experienced physical stress-induced changes (Kumari et al., 2018). Lebranche mullet and marbled spinefoot both lost visual indicators of consciousness in under 3 min using 70 mg/L clove oil and demonstrated no aversive behavior (da Silva Braz et al, 2017; Ghanawi et all., 2011). Additional research should be conducted using EEG analysis to ensure that fish are being rendered unconscious and not just immobilized when exposed to clove oil. The effectiveness and safety of clove oil should also be assessed as the lack of aversive behavior by all fish suggests there is great potential for being a humane method of stunning fish.

4.8 Conclusion

Asphyxia and exsanguination, carbon dioxide and live chilling all require long induction times, during which most species of fish exhibit aversive and stressed behavior. Though easy and relatively inexpensive to implement, it is apparent that these stunning methods are not the most humane and should not be an encouraged practice. Percussive and electrical stunning cause an immediate loss of visual indicators of consciousness when successfully implemented but run the risk of being ineffective and stressful when improper force or current is used. Clove oil appears to have the most potential as a humane method of stunning. Though not yet approved as a stunning method in Europe, clove oil appears to be an inexpensive and low stress stunning method capable of stunning large groups of fish quickly and efficiently. However, this method requires further research on consciousness during stunning and on specie specific concentrations.

Behavior displayed during or after the stunning process can be used as indicators that a method is stressful for fish, provided the fish is not immobilized. However, measuring the plasma cortisol concentrations pre- and post-stun can provide a more quantifiable method of assessing stress levels in fish. While the loss of visual indicators in fish post-stun is a popular method of assessing consciousness, it is not uncommon for fish to maintain brain activity past the point when indicators are lost. The use of EEG and visual evoked responses (VERs) to analyze brain activity in fish is a more accurate and reliable method of measuring consciousness.

With aquaculture growing quickly as a food production sector, it is important that the stunning and killing methods be optimized. Not only will development of humane stunning and killing methods increase the welfare of fish, it could also reduce the number of fish being discarded due to damage from improper methods. The large number of subtropical and tropical species in aquaculture without information on humane stunning methods, while unfortunate, is a wealth of opportunities for researchers hoping to make a difference in the world of fish welfare.

Table 1: Tropical and subtropical fish with a production volume exceeding 350 000 tonnes in aquaculture in 2016 according to FAO (2018a).

Scientific name	Latitudinal Range	Tonnes produced (2016)	Est. weight range (in kg)	Est. fish harvested (in millions)
Grass carp (*Ctenopharyngodon idellus*)	65°N-25°N	6 068 015	0.5-2.5[a]	2 400-12 100
Silver carp (*Hypophthalmichthys molitrix*)	64°N-43°S	5 300 736	0.3-1.5[a]	3 500-17 700
Common/European carp (*Cyprinus carpio*)	60°N-22°N	4 556 622	0.5-2.5[a]	1 800-9 100
Nile tilapia (*Oreochromis niloticus*)	35°N-5°S	4 199 567	0.25-0.8[a]	5 200-16 800
Bighead carp (*Hypophthalmichthys nobilis*)	64°N-18°S	3 526 812	0.5-1.5[a]	2 300-7 000
Catla (*Gibelion/Catla catla*)	34°N-19°S	2 960 554	0.3-2.0[a]	1 500-9 900
Roho labeo (*Labeo rohita*)	32°N-21°S	1 843 496	0.3-1.5[a]	1 200-6 100
Milkfish (*Chanos chanos*)	46°N-52°S	1 188 082	0.25-0.5[a]	2 400-4 700
Wuchang bream (*Megalobrama amblycephala*)	32°N-23°N	826 178	0.45-0.5[a]	1 600-1 800
Black carp (*Mylopharyngodon piceus*)	53°N-15°N	632 055	2.0-3.0[a]	210-315
Snakehead (*Channa argus*)	54°N-25°N	518 207	0.35-1.0[b]	520-1 500
Striped catfish (*Pangasius hypophthalmus*)	19°N-8°N	515 054	0.5-1.5[a]	340-1 000
Mrigal carp (*Cirrhinus mrigala*)	30°N-0°N (est.)	479 680	1.0-2.0[c]	240-480
Amur catfish (*Silurus asotus*)	53°N-23°N	458 356	1.2-4.5[d]	100-380
Channel catfish (*Ictalurus punctatus*)	55°N-25°N	432 932	0.34-0.68[a]	635-1 300
Yellow catfish (*Pelteobagrus fulvidraco*)	55°N-15°N	417 347	0.3[e]	1 400
Pond loach (*Misgurnus anguillicaudatus*)	53°N-27°S	401 203		
Asian swamp eel (*Monopterus albus*)	34°N-6°S	386 179	0.45[f]	860
Largemouth black bass (*Micropterus salmoides*)	46°N-24°N	376 070	0.2-8.0[g]	45-1 900
Total:		**34 316 970**		**26 250-94 335**

Sources: [a] Mood and Brooke, 2012, [b] Rosmawati et al., 2018, [c] Modern Farming Methods, 2018, [d] Petr, 1999, [e] FishBase, 2018, [f] Allen, 2018, [g] Texas Parks & Wildlife, 2018

Table 2: Summary of stunning and killing methods during slaughter reviewed in this report

		Consciousness		Cortisol	Notes	Source
		Lost Visual/EEG	**Returned Visual/EEG**	**(ng*ml^{-1}) Control/post stun**		
Percussion and Spiking						
Silver carp	manual percussion	0/-	-/-	-/-	-	Zhang et al., 2017
Common carp	manual percussion	0/-	0.5-30 min/-	5-15/228±100	visual indicators present in 46% of carp at time of slaughter	Retter et al., 2018
-	60 g captive bolt, 7.5 bar pressure	0/0	Indefinite	-/-	2 out of 22 carp responded to pain stimuli 0.5 min after post stun	Lambooij et al., 2007
Nile Tilapia	manual percussion	0/-	-/-	20/412±63	-	Wang et al., 2017
African sharptooth catfish	16 mm captive needle, 3 bar pressure	0/16±17s	Indefinite	-/-	slow muscle cramps for 2 ± 3 s after stun	Lambooij et al., 2003
Gilthead seabream	pneumatic gun, 6 bar pressure	0/0	-/-	-/-	-	Van de Vis et al., 2003
European eel	16 mm captive needle, 3 bar pressure	0/11±8 s	-/-	-/-	Some had slow muscle cramps lasting >1h post stun	Lambooij et al., 2002a
Electrical						
Grass carp	wet, freshwater containing 2 g NaCl/L, 1.6 m between electrodes, 3 A / 200 V for 1 min followed by 3.5 A / 22 V for 2 min	0/-	-/-	-/-	-	Scherer et al., 2005
Common carp	wet, conductivity of 337-1179 µS/cm, 36-47 V / 0.0213-0.0276 A for 1-6 min	0/-	0.5-30 min/-	5-15/114±77	Visual indicators present in 28% of carp 0.5-2.5 min post-stun.	Retter et al., 2018
-	wet, conductivity of 441-959 µS/cm, 29-54 V / 0.038-0.1464 A for 0.5-5 min followed by percussion	0/-	0.5-30 min/-	5-15/151±106	Visual indicators present in 12% of carp 0.5-30 min post-stun.	Retter et al., 2018
-	wet, conductivity of 600 µS/cm, 0.09-0.41 A/dm^2 / 15.5-68.2 V/dm for 1-5 min	0/unknown	-/<30 s	-/-	Visual indicators returned long after brain activity	Retter et al., 2018
-	wet, head-only, 300 V / 4.7 mA DC for 3 s	0/-	-/-	5-15/3-800+		Daskalova et al., 2016b
-	wet, freshwater, 16 cm between electrodes, 113 V / 0.14 A/dm^2 AC for 1 s	0/0	-/34±14 s	-/-	-	Lambooij et al., 2007

(Continued)

Table 2 *(Continued)*

		Consciousness		Cortisol	Notes	Source
		Lost Visual/EEG	**Returned Visual/EEG**	**(ng*ml^{-1}) Control/post stun**		
-	wet, freshwater, 16 cm between electrodes, 411 V / 0.73 A/dm^2 for 5 s followed by chilling in ice water	0/0	-/Indefinite	-/-	-	Lambooij et al., 2007
-	dry, head-only, 163 V / 0.23 A / 50 Hz AC for 1 s	0/0	48±8 s/31±14 s	-/-	-	Lambooij et al., 2007
Nile tilapia	wet, top-to-bottom, 16 cm between electrodes, 700 µS/cm conductivity, 1.0 A_{rms}/dm^2 / 50 Hz AC for 1 s	0/0	-/26±10 s	-/-	-	Lambooij et al., 2008
-	wet, head-to-tail, 700 µS/cm conductivity, 0.4 A_{rms}/dm^2 / 50 Hz AC for 1 s	0/0	-/27±10 s	-/-	-	Lambooij et al., 2008
-	wet, side-to-side, 650 µS/cm conductivity, pulsed square wave alternating current 0.6 A_{rms}/dm^2 / 133 Hz / 43% duty cycle for 1 s	0/0	-/51±37 s	-/-	-	Lambooij et al., 2008
-	wet, 49 cm between electrodes, 700 µS/cm conductivity, 154 V / 50 Hz / 8 A for 3 min	30 s/-	-/-	~20/15	-	Oliveira Filho et al., 2015
-	electrofishing, 200 V AC	-/-	-/-	-/-	tremors and fine blood spots on the skin	Mahmoud et al., 2019
Channel catfish	400 V AC or DC	-/-	-/-	-/-	convulsions post-stun	Boggess et al., 1973
African sharptooth catfish	dry, head only, 362 ± 32 V / 629 ± 180 mA applied for 1.2 s	-/-	-/23±8 s	-/-	-	Lambooij et al., 2004
-	wet, top-to-bottom, 16cm between electrodes, conductivity of 876 µS/cm, 291 ± 5 V / 1.60 ± 0.11 A	-/-	-/28±8 s	-/-	-	Lambooij et al., 2006
European seabass	wet, Fishkill® EG200 equipment, 40 V / 50 Hz for 4 min	0/-	-/-	-/-	-	Zampacavallo et al., 2015
-	wet, Fishkill® EG200 equipment, 120 V / 400 Hz for 1 min followed by 40 V /50 Hz for 3 min	0/-	-/-	-/-	-	Zampacavallo et al., 2015
Gilthead seabream	dry, head-only, 80 V / 50 Hz / 27-450 mA AC for 1 s	-/0	-/37 s	-/-	only 1 of 10 was stunned with this method	Van de Vis et al., 2003
-	dry, head-only, 80 V / 50 Hz / 400+ mA AC for 10 s	-/0	-/16 s-indefinite	-/-	9 of 10 was stunned with this method, 3 fish recovered VERs, 6 did not recover	Van de Vis et al., 2003

(Continued)

Table 2 *(Continued)*

		Consciousness		Cortisol	Notes	Source
		Lost Visual/EEG	**Returned Visual/EEG**	**(ng*ml^{-1}) Control/post stun**		
Cobia	wet, pulsed direct current 100 V / 30 Hz / 25% duty cycle for 5 s	0/-	36 ±6 s/-	25/375	body tensing, opercular flaring, fin extension	Trushenski et al., 2012
-	wet, 49 cm between electrodes, 700 µS/cm conductivity, 150 V / 60 Hz / 7.3 A AC for 3 min	0/-	-/-	-/-	-	Baldi et al., 2018
Pacu	wet, freshwater, Smith Root LR-24 backpack electrofisher, 205 V / 50 Hz / 1.3 A / duty cycle of 70% for 45 s	0/-	62 ±13 s/-	-/-	-	Rucinque et al., 2018
-	wet, freshwater, Smith Root LR-24 backpack electrofisher , 400 V / 30 Hz / 0.9 A / duty cycle of 30% for 30 s	0/-	50 ±10 s/-	-/-	-	Rucinque et al., 2018
-	wet, 49 cm between electrodes, 700 µS/cm conductivity, 200 V / 50 Hz AC for 3 min	0/-	4 min/-	-/-	no signs of visible stress, minimal internal bleeding	Oliveira Filho et al., 2015
European eel	dry, head only, 255 ± 4 V / 50 Hz / 545 ± 32 mA AC for 1s	0/0	-/38±25 s	-/-	-	Lambooij et al., 2002c
-	wet, 500 µS/cm conductivity, 200 V, 0.636 ± 0.040 A_{rms}/dm^2 for 1.6 ± .4 s	0/0	-/34 s	-/-	-	Lambooij et al., 2002d
-	wet, 500 µS/cm conductivity, 196 ± 2 V / 0.693 ± 0.011 A/dm^2 for 1 s followed by 50 V / 0.165 A/dm^2 for 5 min in nitrogen saturated water	-/-	-/-	-/-	No brain activity seen post stun but 1 eel was able to right itself after 60 min while non-responsive to needle scratch	Lambooij et al., 2002d
-	wet, 15cm between electrodes, 500 µS/cm conductivity, 250 V for 10 s followed by 80 V for 7 min in nitrogen saturated water	-/-	-/-	-/-	Some showed muscular activity or breathing post-stun	Morzel & Van de Vis, 2003
-	dry, head-only, 50+ V_{rms}, / 50 Hz / 0.12-0.24 A_{rms} for 1 s	-/8-10 s	-/22-32 s	-/-	-	Robb et al., 2002
-	dry, head-only, 65 V, 50 Hz / 0.06-0.96 A for 30 s	-/-	160 s-indefinite/-	-/-	no recovery	Robb et al., 2002

(Continued)

Table 2 *(Continued)*

		Consciousness		Cortisol	Notes	Source
		Lost Visual/EEG	**Returned Visual/EEG**	**(ng*ml^{-1}) Control/post stun**		
Common sole	dry, tail-first, 1.22 ± 0.68 A_{rms} / 152 ± 0.5 V_{rms} for 1 s	-/-	-/<1 s	-/-	non-responsive to needle scratch 1 min after stun, but EEG shows fish were conscious	Daskalova et al., 2016a
-	dry, tail-first, 1.20 ± 0.59 A_{rms} / 152 ± 0.5 V_{rms} followed by 0.36 ± 0.15 A_{rms} for 19 s followed by ice chilling	0/-	-/5 min	-/-	some responded to scratch within 5 min post-stun, no fish recovered after 75 min	Daskalova et al., 2016a
-	dry, 0.65 ± 0.23 A_{rms}, 100 Hz for 1 s	-/-	30 s/23±10 s	-/-	-	Llonch et al., 2012
-	dry, 0.65 ± 0.23 A_{rms}, 100 Hz for 5 s followed by ice chilling	0/-	30 s/23±22 s	-/-	-	Llonch et al., 2012
Carbon dioxide						
Common carp	pH 4.1, 16°C water	10 min/-	-/-	-/-	escape attempts, aversive behavior	Rahmanifarah et al., 2011
-	submersion in water with dissolved CO_2	-/-	-/-	5-15/633-800+	-	Varga et al., 2014
Nile tilapia	pH 4.2, 21.6°C water	30 min/-	-/-	20/17	no aversive behavior, air intake at surface of water	Oliveira Filho et al., 2015
Channel catfish	Submersion in water with dissolved CO_2	3 min/-	-/-	-/-	-	Boggess et al., 1973
European seabass	submersion in water saturated with CO_2, 9.5 °C	16 min/-	-/-	60/~350	-	Acerete et al., 2009
Cobia	736 ± 21 mg/L, 27 °C water	2.7 min/-	1 min/-	40/450	-	Trushenski et al., 2012
-	pH 4.5, 23.4°C water	48/min/-	-/-	-/-	violent escape attempts, aversive behavior	Baldi et al., 2018
Nitric oxide						
Nile tilapia	solubility of NO was 136 ml/1 L water at 20 °C	20 min/-	-/-	20/303±44	no aversive behavior	Wang et al., 2017

(Continued)

Table 2 *(Continued)*

		Consciousness		Cortisol	Notes	Source
		Lost Visual/EEG	**Returned Visual/EEG**	**(ng*ml^{-1}) Control/post stun**		
Clove oil						
Common carp	1 ml/L, 21°C water	225 s/-			No aversive behavior	Rahmanifarah et al., 2011
-	40 ppm, 27°C water	3 min/-	4.6 min/-	-/-	-	Bosworth et al., 2007
-	50 ppm, 27°C water	3 min/-	13.2 min/-	-/-	-	Bosworth et al., 2007
-	100 ppm, 27°C water	3 min/-	Indefinite/-	-/-	-	Bosworth et al., 2007
Channel catfish	200 ppm, 26°C water for 30 min	-/-	-/-	<10/<10	-	Small, 2003
Gilthead seabream	55 mg/L, 15°C water	3 min/-	4 min/-	-/-	-	Mylonas et al., 2005
Barramundi	25 mg/L AQUI-S, 18-20 °C water	-/-	-/-	1/43	-	Wilkinson et al., 2008
Meagre	30 mg/L, 18°C water	>15 min/-	< 5 min/-	<10/~40	Not all fish lost visual indicators of consciousness	Cárdenas et al., 2016
-	40 or 50 mg/L, 18°C water	< 3 min/-	< 5 min/-	-/-	-	Cárdenas et al., 2016
-	25 or 40 mg/L, 18°C water	>10 min/-	-/-	-/-	Failed to induce anesthesia	Barata et al., 2016
-	55 mg/L, 18°C water	4.5-10 min/-	6-11 min/-	-/-		Barata et al., 2016
-	70 mg/L, 18°C water	2-4 min/-	4-11 min/-	-/-		Barata et al., 2016
-	85 mg/L, 18°C water	2-3 min/-	5-8 min/-	-/-		Barata et al., 2016
Senegal sole	1 ml/L, 16°C water	22 min/-	10 min/-	1/~5	-	Ribas et al., 2007
Spotted snakehead	50 µl/L, 25 ± 2°C water	22 min/-	10 min/-	-/-	-	Kumari et al. 2018
-	100 µl/L, 25 ± 2°C water	9 min/-	13 min/-	-/-	-	Kumari et al. 2018
-	200 µl/L, 25 ± 2°C water	4 min/	17 min/-	-/-	stress-induced changes in organs such as gills and buccal epithelium	Kumari et al. 2018
Lebranche mullet	50 mg/L, 24 ± 1°C water	3.4 min/-	1.8 min/-	-/-	-	Da Silva Braz et al., 2017
-	70 mg/L, 24 ± 1°C water	2.8 min/-	1.2 min/-	-/-	-	Da Silva Braz et al., 2017
-	90 mg/L, 24 ± 1°C water	2.7 min/-	1.1 min/-	-/-	-	Da Silva Braz et al., 2017
-	110 mg/L, 24 ± 1°C water	2 min/-	1.1 min/-	-/-	-	Da Silva Braz et al., 2017
Marbled spinefoot	70 mg/L, 23 ± 1°C water	~1 min/-	<5 min/-	-/-	-	Ghanawi et al., 2011
-	70 mg/L, 20°C water	1.4 min/-	5.3 min/-	-/-	-	Santos et al., 2015
-	70 mg/L, 30°C water	0.9 min/-	3.7 min/-	-/-	-	Santos et al., 2015

(Continued)

Table 2 *(Continued)*

		Consciousness		Cortisol	Notes	Source
		Lost Visual/EEG	**Returned Visual/EEG**	**(ng*ml^{-1}) Control/post stun**		
Live chilling						
Grass carp	1:1.5 ratio ice:water for 20 min	10 min/-	-/-	-/-	"strong aversive behavior"	Scherer et al., 2005
Silver carp	1:1 ratio ice:water for 50 min	50 min/-	-/-	-/-	decreased swimming attempts, minor escape attempts and irregular ventilation	Zhang et al., 2017
Common carp	ice slurry (0.6 - 1.8°C) for 50 min	48/-	-/-	-/-	aversive swimming, tremors, and escape attempts	Rahmanifarah et al., 2011
-	ice slurry	-/-	-/-	5-15/275	-	Varga et al., 2014
Nile tilapia	1:1 ratio ice:water	20/-	-/-	~20/22	-	Oliveira Filho et al., 2015
African sharptooth catfish	ice water (0.1 ± 0.5°C) for 30 min	5-20 /20 min	-/-	-/-	tachycardia, some fish were conscious but not responsive to noxious stimuli	Lambooij et al., 2006
European seabass	1:2 ratio ice:water for 30 min	20-30 min/-	-/-	-/-	-	Zampacavalo et al., 2015
-	chilled water (1.4 ± 1°C), uncrowded	20 min/-	-/-	-/-	-	Bagni et al., 2007
-	chilled water (1.4 ± 1°C), crowded	45 min/-	-/-	-/-	-	Bagni et al., 2007
-	1:1 ratio ice:water (2 - 4°C)	34 min/-	-/-	60/~360	-	Acerete et al., 2009
-	1:2 ratio ice:water with dissolved gas (70% N_2 + 30% CO_2)	14-19 min/-	-/-	-/-	-	Zampacavalo et al., 2015
-	1:2 ratio ice:water with dissolved gas (40% N_2 + 60% CO_2)	10 min/-	-/-	-/-	-	Zampacavalo et al., 2015
Gilthead seabream	3:1 ratio ice:water (0.8 ± 0.2°C)	4 /5.5 min	-/-	-/-	vigorous movements	Van de Vis et al., 2003
-	ice slurry (1.4°C), uncrowded	20 min/-	-/-	-/-	extreme struggling	Bagni et al., 2007
-	ice slurry (1.4°C), crowded	40min/-	-/-	-/-	extreme struggling	Bagni et al., 2007
Cobia	1:1 ratio ice:water (1.4°C)	17.5 min/-	-/-	-/-	aversive behavior, increased mucus production, attempts to breathe air	Baldi et al., 2018
European eel	ice slurry (0°C) followed by chilled brine (-18°C)	12±5 min/12±6 min	-/-	-/-	Some eel were responsive at time of transfer	Lambooij et al., 2002b
Senegal sole	transfer from 16°C water to 2-4°C water	-/-	-/-	1/35	-	Ribas et al., 2007

(Continued)

Table 2 *(Continued)*

		Consciousness		Cortisol	Notes	Source
		Lost Visual/EEG	**Returned Visual/EEG**	**(ng*ml^{-1}) Control/post stun**		
Asphyxiation						
Common carp	netted and held in air for 90±15 min	-/-	-/-	5-15/634-800+	-	Daskalova et al., 2016b
-	netted and held in air	293 min/-	-/-	-/-	violent scramble	Rahmanifarah et al., 2011
Nile tilapia	netted and held in air	30 min/-	-/-	-/-	rapid breathing, gasping, vigorous movement, lesions and loss of scales	Mahmoud et al., 2019
Channel catfish	asphyxia on ice, 1.7°C for 3 h	-/-	-/-	-/-	gall bladders enlarged	Boggess et al., 1973
European seabass	placed in bucket exposed to air	34/-	-/-	60/~480	-	Acerete et al., 2009
-	placed in bucket exposed to air, uncrowded	35 min/-	-/-	-/-	-	Bagni et al., 2017
-	placed in bucket exposed to air, crowded	70 min/-	-/-	-/-	-	Bagni et al., 2017
Gilthead seabream	placed in bucket exposed to air, uncrowded	25 min/-	-/-	-/-	-	Bagni et al., 2017
-	placed in bucket exposed to air, crowded	50 min/-	-/-	-/-	-	Bagni et al., 2017
-	asphyxia in air	4/5 min	-/-	-/-	escape attempts	Van de Vis et al., 2003
Senegal sole	asphyxia on ice	-/-	-/-	1/263	-	Ribas et al., 2007
Exsanguination						
Silver carp	gill cutting	40 min/-	-/-	-/-	severe bleeding, no escape behavior	Zhang et al., 2017
-	gill cutting	-/-	-/-	-/-	severe struggling and aversive behavior	Zhang et al., 2017
African sharptooth catfish	gill cutting	15 min/-	-/-	-/-	-	Lambooij et al., 2004
-	gill cutting after recovery from head only electrical stunning	5 min/ 12±6 s	-/-	-/-	-	Lambooij et al., 2004
European eel	decapitation, head in water	25-55 min/-	-/-	-/-	respiratory movements and opening of the mouth	Verheijen & Flight 1997
-	decapitation, head in air	1.25-7.75 h	-/-	-/-	Respiratory-, twisting- pectoral fin-movements and opening of the mouth	Verheijen & Flight 1997

5 References

Acerete, L., Reig, L., Alvarez, D., Flos, R., and Tort, L. (2009). "Comparison of two stunning/slaughtering methods on stress response and quality indicators of European sea bass (*Dicentrarchus labrax*)". In: *Aquaculture* 287.1-2, pp. 139-144.

Allen, U. (2018). *Monopterus albus (Asian swamp eel)*. [online] CABI: Invasive Species Compendium. Available at: https://www.cabi.org/isc/datasheet/74114 [Accessed 10 Dec. 2018].

Anderson, W. G., Mckinley, R. S., and Colavecchia, M. (1997). "The Use of Clove Oil as an Anesthetic for Rainbow Trout and Its Effects on Swimming Performance". In: *North American Journal of Fisheries Management* 17.2, pp. 301–307.

Anonymous (1995). Operating manual for the product certification schemes for Scottish quality farmed salmon and smoked Salmon Scottish quality salmon. Iverness, Scotland.

Aqui-s.com. (2018). *AQUI-S*. [online] Available at: https://www.aqui-s.com/aqui-s-products/aqui-s [Accessed 12 Dec. 2018]

Auperin, B., Baroiller, J. F., Ricordel, M. J., Fostier, A., and Prunet, P. (1997). "Effect of confinement stress on circulating levels of growth hormone and two prolactins in freshwater-adapted tilapia (*Oreochromis niloticus*)". In: *General and Comparative Endocrinology* 108.1, pp. 35-44.

Bagni, M., Civitareale, C., Priori, A., Ballerini, A., Finoia, M., Brambilla, G., and Marino, G. (2007). "Pre-slaughter crowding stress and killing procedures affecting quality and welfare in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*)". In: *Aquaculture* 263.1-4, pp. 52-60.

Baldi, S. C. V., Parisi, G., Bonelli, A., Balieiro, J. C. C., Guimarães, J. L., and Viegas, E. M. M. (2018). "Effects of different stunning/slaughter methods on frozen fillets quality of cobia (*Rachycentron canadum*)". In: *Aquaculture* 486.3, pp. 107-113.

Barata, M., Soares, F., Aragão, C., Almeida, A. C., Pousão-Ferreira, P., & Ribeiro, L. (2016). "Efficiency of 2-phenoxyethanol and Clove Oil for Reducing Handling Stress in Reared Meagre, *Argyrosomus regius* (Pisces: Sciaenidae)". In: *Journal of the World Aquaculture Society* 47.1, pp. 82-92.

Barreto, R. E. and Volpato, G. L. (2004). "Caution for using ventilatory frequency as an indicator of stress in fish". In: *Behavioural Processes* 66.1, pp. 43-51.

Barton, B. A. (2002). "Stress in Fishes: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids". In: *Integrative and Comparative Biology* 42.3, pp.517-525.

Barton, B. A. and Iwama, G. K. (1991). "Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids". In: *Annual Review of Fish Diseases* 1, pp. 3–26.

Boggess, T. S., Heaton, E. K., Shewfelt, A. L., and Parvin, D. W. (1973). "Techniques for stunning channel catfish and their effects on product quality". In: *Food Science* 38.8, pp. 1190-1193.

Bosworth, B. G., Small, B. C., Gregory, D., Kim, J., Black, S., and Jerrett, A. (2007). "Effects of rested-harvest using the anesthetic AQUI-S™ on channel catfish, *Ictalurus punctatus*, physiology and fillet quality". In: *Aquaculture* 262.2-4, pp. 302-318.

Cárdenas, C:, Toni, C., Martos-Sitcha, J. A., Cárdenas, S., de las Heras, V., Baldisserotto, B., Heinzmann, B.M., Vázques, R., and Mancera, J. M. (2016) "Effects of clove oil, essential oil of Lippia alba and 2-phe anaesthesia on juvenile meagre, *Argysomus regius* (Asso, 1801)". In: *Journal of Applied Ichthyology* 32.4, pp 693-700

da Silva Braz, R., de Oliveira Silva, I., Tesser, M., Sampaio, L., and Rodrigues, R. (2017). ”Benzocaína, MS-222, eugenol e mentol como anestésicos para juvenis de tainha Mugil liza.” In: *Boletim do Instituto de Pesca* 43. 605-613.

Daskalova, A. H., Bracke, M. B.M., Vis, J. W. van de, Roth, B., Reimert, H. G.M., Burggraaf, D., and Lambooij, E. (2016a). “Effectiveness of tail-first dry electrical stunning, followed by immersion in ice water as a slaughter (killing) procedure for turbot (*Scophthalmus maximus*) and common sole (Solea solea)”. In: *Aquaculture* 455, pp. 22–31.

Daskalova, A., Pavlov, A., Kyuchukova, R., and Daskalov, H. (2016b). “Humane slaughter of carp – A comparison between three stunning procedures”. In: *Turkish Journal of Fisheries and Aquatic Sciences* 16.4, pp. 753-758

FAO (2018a). FAO Yearbook. Fishery and Aquaculture Statistics 2016. Tech. rep. Rome: Food and Agriculture Organization of the United Nations. p.104.

FAO (2018b). *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals*. Tech. rep. Rome: Food and Agriculture Organization of the United Nations, p. 211.

FAO (2018c). FAO Fisheries & Aquaculture Global Aquaculture Production, Overview. [online] Available at: http://www.fao.org/fishery/statistics/global-aquaculture-production/en [Accessed 13 Dec. 2018].

Fanouraki, E., Mylonas, C. C., Papandroulakis, N., and Pavlidis, M. (2011). “Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture”. In: *General and Comparative Endocrinology* 173, pp. 313-322.

Ghanawi, J., Monzer, S., and Saoud, I. P. (2011). “Anaesthetic efficacy of clove oil, benzocaine, 2-phenoxyethanol and tricaine methanesulfonate in juvenile marbled spinefoot (*Siganus rivulatus*)”. In: *Aquaculture Research* 44.3, pp. 359-366.

Gräns, A., Niklasson, L., Sandblom, E., Sundell, K., Algers, B., Berg, C., Lundh, T., Axelsson, M., Sundh, H., and Kiessling, A. (2016). “Stunning fish with CO_2 or electricity: contradictory results on behavioural and physiological stress responses”. In: *Animal* 10.2, pp. 294-301

Grimsbø, E. (2016). “Measuring methods for fish welfare during slaughter based on electrical impedance, EEG, ECG and blood parameters”. PhD thesis. University of Bergen, p. 63.

Hayashi, M. (2003). “Temperature-electrical conductivity relation of water for environmental monitoring and geophysical data inversion”. In: *Environmental Monitoring and Assessment* 96. pp. 119-128.

Hikasa, Y., Takase, K., Ogasawara, T., and Ogasawara, S. (1986). “Anesthesia and Recovery with Ticaine Methanesulfonate, Eugenol and Thiopental Sodium in the Carp, *Cyprinus carpio*”. In: *Nihon Juigaku Zasshi* 48.2, pp. 341–351.

Huidobro, A., Pastor, A., and Tejada, M. (2000). “Quality index method developed for raw gilthead seabream (*Sparus aurata*)”. In: *Journal of Food Science* 65.7, pp. 1202-1205

“Ethics and the politics of food” (2006). In: *6th Congress of the European Society for Agricultural and Food Ethics*. Ed. by Matthias Kaiser and Marianne Lien. Wageningen: Wageningen Academic Publishers, p. 592.

Kestin, S. C., Vis, J. W. van de, and Robb, D. H. F. (2002). “Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them”. In: *Veterinary Record* 150, pp. 302–307.

Kestin, S., Wotton, S., and Adams, S. (1995). “The effect of CO_2, concussion or electrical stunning of rainbow trout (Oncorhynchus mykiss) on fish welfare”. In: *Quality in Aquaculture*. International Conference Aquaculture Europe, Trondheim, Norway, pp. 380–381.

Kestin, S., Wotton, S., and Gregory, N. (1991). “Effect of slaughter by removal from water on visual evoked activity in the brain and reflex movement of rainbow trout (*Oncorhynchus mykiss*)”. In: *The Veterinary Record* 128.19, pp. 443-446

Kugino, K., Tamaru, S., Hisatomi, Y., and Sakaguchi, T. (2016). “Long-duration carbon dioxide anesthesia of fish using ultra fine (nano-scale) bubbles”. In: *PLoS ONE* 11.4, p. 9.

Kumari, P., Abidi, S., & Parwez, I. (2018). “Evaluation of anaesthetizing efficacy of clove oil in *Channa punctatus*”. In: *Article in Turkish Journal of Fisheries and Aquatic Sciences* 6.5, pp. 262-266.

Lambooij, E., Gerritzen, M. A., Reimert, H., Burggraaf, D., and Vis, J. W. van de (2008). “A humane protocol for electro-stunning and killing of Nile tilapia in fresh water”. In: *Aquaculture* 275.1-4, pp. 88-95.

Lambooij, E., Grimsbø, E., Vis, J. W van de, Reimert, H. G. M., Nortvedt, R., and Roth, B. (2010). “Percussion and electrical stunning of Atlantic salmon (*Salmo salar*) after dewatering and subsequent effect on brain and heart activities”. In: *Aquaculture* 300.1-4, pp. 107–112.

Lambooij, E., Kloosterboer, R. J., Gerritzen, M. A., and Vis, J. W. van de (2004). “Head-only electrical stunning and bleeding of African catfish (*Clarias gariepinus*): assessment of loss of consciousness”. In: *Animal Welfare* 13.1, pp. 71–76.

Lambooij, E., Kloosterboer, R. J., Gerritzen, M. A., and Vis, J. W. van de (2006). “Assessment of electrical stunning in fresh water of African Catfish (*Clarias gariepinus*) and chilling in ice water for loss of consciousness and sensibility”. In: *Aquaculture* 254.1-4, pp. 388–395.

Lambooij, E., Kloosterboer, R. J., Pieterse, C., Gerritzen, M. A., and Van De Vis, J. W. (2003). “Stunning of farmed African catfish (*Clarias gariepinus*) using a captive needle pistol; assessment of welfare aspects”. In: *Aquaculture Research* 34.14, pp. 1353–1358.

Lambooij, E., Pilarczyk, M., Bialowas, H., Boogaart, J. G M van den, and Vis, J. W. van de (2007). “Electrical and percussive stunning of the common carp (*Cyprinus carpio L.*): Neurological and behavioural assessment”. In: *Aquacultural Engineering* 37.2, pp. 171–179.

Lambooij, E., Van de Vis, J. W., Kloosterboer, R. J., and Pieterse, C. (2002a). “Evaluation of captive needle stunning of farmed eel (*Anguilla anguilla L.):* Suitability for humane slaughter”. In: *Aquaculture* 212.1-4, pp. 141-148.

Lambooij, E., Van de Vis, J. W., Kloosterboer, R. J., and Pieterse, C. (2002b). “Welfare aspects of live chilling and freezing of farmed eel (*Anguilla anguilla L.):* Neurological and behavioural assessment”. In: *Aquaculture* 210.1-4, pp. 159-169.

Lambooij, E., Van de Vis, J. W., Kloosterboer, R. J., and Pieterse, C. (2002c). ”Evaluation of head-only and head to tail electrical stunning of farmed eels (*Anguilla anguilla L*) for development of a humane slaughter method)”. In: *Aquaculture Research* 33.5, pp. 323-331

Lambooij, E., Van De Vis, J. W., Kuhlmann, H., Münkner, W., Oehlenschläger, J., Kloosterboer, R. J., and Pieterse, C. (2002d). “A feasible method for humane slaughter of eel (*Anguilla anguilla L.*): Electrical stunning in fresh water prior to gutting”. In: *Aquaculture Research* 33.9, pp. 643–652.

Llonch, P., Lambooij, E., Reimert, H. G. M., and Vis, J. W. van de (2012). “Assessing effectiveness of electrical stunning and chilling in ice water of farmed yellowtail kingfish, common sole and pikeperch”. In: *Aquaculture* 264-385, pp. 143–149.

Mahmoud, M. A., Mansour, H. A., Abdelsalam, M., AbuBakr, H. O., Aljuaydi, S. H., and Afify, M. (2019). “Evaluation of electrofishing adopted by Egyptian fish farmers”. In: *Aquaculture* 498, pp.380-387.

Marx, H., Brunner, B., Weinzierl, W., Hoffmann, R., and Stolle, A. (1997). "Methods of stunning freshwater fish: impact on meat quality and aspects of animal welfare". In: *Z Lebensm Unters Forsch A* 204.4, pp. 282–286.

Matthess, G. (1982). *The Properties of Groundwater*, John Wiley & Sons, New York

Modern Farming Methods. (2018). *Mrigal Fish Information | Modern Farming Methods*. [online] Available at: https://www.roysfarm.com/mrigal-fish/ [Accessed 10 Dec. 2018]

Morzel, M. and Van De Vis, H. (2003). "Effect of the slaughter method on the quality of raw and smoked eels (*Anguilla anguilla L.*)" In: *Aquaculture Research* 34.1, pp. 1-11.

Mylonas, C. C., Cardinaletti., Sigelaki, I., and Polzonetti-Magni, A. (2005). "Comparative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass (Dicentrarchus labrax) and gilthead sea bream (*Sparus aurata*) at different temperatures". In: *Aquaculture* 246, pp. 467-481.

OIE (2018). *Introduction to the Recommendations for Animal Welfare*. Tech. rep. Paris, France. Chap. 7, p. 4.

Oliveira Filho, P. R. C. de, Montes Girao, P. J., Lapa-Guimarães, J., Natori, M. M., Vargas, S. C., and Viegas, E. M. M. (2016). "Impact of electrical stunning on fish behavior and meat quality of pacu (*Piaractus mesopotamicus*)". In: *Acta Scientiarum. Technology* 38.1, pp. 81-88.

Oliveira Filho, P. R. C. de, Montes Girao, P. J., Melo, M., and Viegas, E. M. M. (2015). "Indicators of stress in tilapia subjected to different stunning methods". In: *Bol. Inst.* Pesca 41.2, pp. 335-343.

Petr, T. (1999). *Fish and fisheries at higher altitudes*. Rome: FAO, p.223.

Poli, B. M., Parisi, G., Scappini, F., and Zampacavallo, G. (2005). "Fish welfare and quality as affected by pre-slaughter and slaughter management". In: *Aquaculture International* 13, pp. 29–49.

Quick, I. A., and Laming, P. R. (1990). "Relationship between ecg, eeg, and sps responses during arousal in the goldfish (*Carassius auratus*)". In: *Comparative Biochemistry and Physiology* 95.3, pp. 459-471.

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". In: *Turkish Journal of Fisheries and Aquatic Sciences* 11.1, pp. 135–143.

Readman, G. D. (2015). "Advances in anaesthetics for fish: greater understanding of species specific welfare associated with induction and efficacy". PhD thesis. University of Bristol, p. 332.

Retter, K., Esser, K., Lüpke, M., Hellmann, J., Steinhagen, D., and JungSchroers, V. (2018). "Stunning of common carp: Results from a field and a laboratory study". In: *BMC Veterinary Research* 14.205, pp. 1–11.

Ribas, L., Flos, R., Reig, L., MacKenzie, S., Barton, B. A., and Tort, L. (2007). "Comparison of methods for anaesthetizing Senegal sole (*Solea senegalensis*) before slaughter: Stress responses and final product quality". In: *Aquaculture* 269.1-4, pp. 250-258.

Robb, D. H. F. and Kestin, S. C. (2002). "Methods used to kill fish: Field observations and literature reviewed". In: *Animal Welfare* 11, pp. 269–282.

Robb, D. H. F., Wotton, S. B., McKinstry, J. L., Sorensen, N. K., Kestin, S. C., and Sorensen, N. K. (2000). "Commercial slaughter methods used on Atlantic salmon: determination of the onset of brain failure by electroencephalography". In: *Veterinary Record* 147.11, pp. 298–303.

Robb, D. H. F., Wotton, S. B., and Van De Vis, J. W. (2002). "Preslaughter electrical stunning of eels". In: *Aquaculture Research* 33.1, pp. 37-42.

Rosmawati, E. A., Tawali, A., Said, M. and Sari, D. (2018). “Effect of body weight on the chemical composition and collagen content of snakehead fish Channa striata skin”. In: *Fisheries Science* 84.6, pp. 1081-1089.

Ross, L. G., Ross, B., and Ross, B. (2009). *Anaesthetic and Sedative Techniques for Aquatic Animals: Third Edition*. 3rd. Oxford: Wiley, Blackwell Publishing, p. 228.

Rucinque, D. S., Watanabe, A. L., and Molento, C. F. M. (2018). “Electrical stunning in pacu (Piaractus mesopotamicus) using direct current waveform”. In: *Aquaculture* 497, pp. 42–48.

Santos, S., Ghanawi, J., and Saoud, I. P. (2015). “Effects of water temperature and body weight on anaesthetic efficiency in marbled rabbitfish (*Siganus rivulatus*)”. In: *Aquaculture Research* 26, pp. 928-936.

Scherer, R., Augusti, P. R., Steffens, C., Bochi, V. C., Heckttheuer, L. H., Lazzari, R., Radünz-Neto, J., Pomblum, S. C. G., and Emanuelli, T. (2005). “Effect of slaughter method on postmortem changes of grass carp (Ctenopharyngodon idella) stored in ice”. In: *Journal of Food Science*. 70.5, pp. 348-353.

Sitnikov, I. G. (2009). “Principal Weather Systems in Subtropical and Tropical Zones”. In: *Environmental Structure and Function: Climate System - Volume 1*. Ed. by G. V. Gruza. Moscow, Russia: EOLSS Publications, p. 207.

Sneddon, L. U. (2015). “Pain in aquatic animals”. In; *Journal of Experimental Biology*, 218.7, pp. 967-976.

Small, B. C. (2003). “Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish Ictalurus punctatus”. In: *Aquaculture* 218.1-4, pp. 177-185.

Subtropics - AMS Glossary (2012). URL: http://glossary.ametsoc.org/wiki/Subtropics.

Texas Parks & Wildlife. (2018). *Estimating Weight of Largemouth Bass*. [online] Available at: https://tpwd.texas.gov/fishboat/fish/recreational/catchrelease/bass_length_weight.phtml [Accessed 10 Dec. 2018].

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”. In: *Journal of Applied Ichthyology* 28.4, pp. 553–564.

Trushenski, J. T., Bowzer, J. C., Bowker, J. D., and Schwarz, M. H. (2012). “Chemical and Electrical Approaches to Sedation of Cobia: Induction, Recovery, and Physiological Responses to Sedation”. In: *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* 4, pp. 639–650.

Van de Vis, H., Kestin, S., Robb, D., Oehlenschläger, J., Lambooij, B., Münkner, W., Kuhlmann, H., Kloosterboer, K., Tejada, M., Huidobro, A., Otterå, H., Roth, B., Sørensen, N. K., Akse, L., Byrne, H., and Nesvadba, P. (2003). “Is humane slaughter of fish possible for industry?” In: *Aquaculture Research* 34.3, pp. 211-220

Varga, D., Szabó, A., and Hancz, C. (2014). “Impact of Handling and Pre-Mortal Stress on the Flesh Quality of Common Carp (*Cyprinus carpio L.)”* In: *The Israeli journal of aquaculture* 22, p. 6.

Verheijen, F. J. and Flight, W. F. G. (1997). “Decapitation and brining: experimental tests show that after these commercial methods for slaughtering eel *Anguilla anguilla (L.*), death is not instantaneous”. In: *Aquaculture research* 28, pp. 361–366.

Wang, Z., Zhao, M. M., Fang, Z., and Chen, D. W. (2017). “Nitric oxide euthanasia: a potential procedure for improving animal welfare and fillet color of tilapia (Oreochromis niloticus)”. In: *Aquaculture International* 25.5, pp. 1845-1856.

Weber, R. A., Pérez-Maceira, J. J., Peleteiro, J. B., García-Martín, L., and Aldegunde, M. (2011). "Effects of acute exposure to 2-phenoxyethanol, clove oil, MS-222 and metomidate on primary and secondary stress responses in Senegalese sole (Solea senegalensis Kaup 1858)". In: *Aquaculture* 321, pp.108-112.

Wilkinson, R. (2012). *Benchmarking harvest methologies in the Australian barramundi aquaculture industry - impacts on stress, product quality and fish welfare*. Tech. rep. Launceston, Tasmania: Australian Maritime College, University of Tasmania, p. 36.

Wilkinson, R., Paton, N., and Porter, M. (2008). "The effects of pre-harvest stress and harvest method on the stress response, rigor onset, muscle pH and drip loss in barramundi (*Lates calcarifer*)". In: *Aquaculture* 282.1-4, pp. 26–32.

Zampacavallo, G., Parisi, G., Mecatti, M., Lupi, P., Giorgi, G., and Poli, B. M. (2015). "Evaluation of different methods of stunning/killing sea bass (*Dicentrarchus labrax*) by tissue stress/quality indicators". In: *Journal of Food Science and Technology* 52.5, pp. 2587-2589.

Zhang, L., Li, Q., Jia, S., Huang, Z., and Luo, Y. (2018). "Effect of different stunning methods on antioxidant status, in vivo myofibrillar protein oxidation, and the susceptibility to oxidation of silver carp (*Hypophthalmichthys molitrix*) fillets during 72h postmortem". In: *Food Chemistry* 246, pp. 121–128.

Zhang, L., Li, Q., Lyu, J., Kong, C., Song, S., and Luo, Y. (2017). "The impact of stunning methods on stress conditions and quality of silver carp (*Hypophthalmichthys molitrix)* fillets stored at 4°C during 72h postmortem". In: *Food Chemistry* 216, pp. 130-137.

6 Appendix

Other tropical and subtropical fish investigated with no stunning information available

Acanthopagrus schlegeli
Ameiurus melas
Anabas testudineus
Anguilla japonica
Arapaima gigas
Astyanax fasciatus
Bagrus bajad
Barbonymus gonionotus
Barbonymus schwanenfeldii
Barbus callensis
Bidyanus bidyanus
Bolbometopon muricatum
Boleophthalmus pectinirostris
Brycon amazonicus
Brycon cephalus
Caranx ignobilis
Carassius auratus
Catla catla
Channa argus
Channa marulius
Channa micropeltes
Channa striata
Chanos chanos
Chitala chitala
Chrysichthys nigrodigitatus
Cichlasoma managuense
Cirrhinus microlepis
Cirrhinus molitorella
Cirrhinus mrigala
Clarias batrachus
Colossoma macropomum
Cromileptes altivelis
Dentex dentex
Dentex gibbosus
Dicentrarchus punctatus
Diplodus puntazzo
Diplodus sargus
Dormitator latifrons
Eleutheronema tetradactylum
Epinephelus areolatus
Epinephelus coioides
Epinephelus fuscoguttatus
Epinephelus lanceolatus
Epinephelus malabaricus
Epinephelus tauvina
Gnathanodon speciosus
Gymnarchus niloticus
Hatcheria macraei
Hemibagrus nemurus
Hemichromis fasciatus
Hepsetus odoe
Heterobranchus bidorsalis
Heterobranchus longifilis
Heteropneustes fossilis
Heterotis niloticus
Hilsa kelee
Hoplosternum littorale
Hypophthalmichthys nobilis
Ichthyoelephas humeralis
Konosirus punctatus
Labeo calbasu
Labeo dussumieri
Labeo rohita
Larimichthys croceus
Lateolabrax japonicus
Lates niloticus
Leptobarbus hoeveni
Lutjanus argentimaculatus
Lutjanus erythropterus
Lutjanus guttatus
Lutjanus johnii
Lutjanus malabaricus
Lutjanus russelli
Megalobrama amblycephala
Micropterus salmoides
Misgumus anguillicaudatus
Monopterus albus
Morone saxatilis
Mugil cephalus
Mylopharyngodon piceus
Notopterus notopterus
Oncorhynchus kisutch
Oncorhynchus tshawytscha
Oreochromis andersonii
Oreochromis aureus
Oreochromis macrochir
Oreochromis mossambicus
Oreochromis shiranus
Oreochromis spilurus
Oreochromis tanganicae
Osphronemus goramy
Osteobrama belangeri
Osteochilus hasselti
Oxyeleotris marmorata
Pagellus bogaraveo
Pagellus erythrinus
Pagrus auratus
Pagrus major
Pagrus pagrus
Pangasius hypophthalmus
Pangasius pangasius
Papyrocranus afer
Paralichthys olivaceus
Pelteobagrus fulvidraco
Piaractus brachypomus
Platax orbicularis
Plecoglossus altivelis
Plectropomus maculatus
Polydactylus sexfilis
Polyodon spathula
Probarbus jullieni
Prochilodus lineatus
Prochilodus mariae
Prochilodus nigricans
Prochilodus reticulatus
Protosalanx hyalocranius
Pseudocaranx dentex
Pseudoplatystoma corruscans
Pseudoplatystoma fasciatum
Pterygoplichthys pardalis
Puntius gonionotus
Puntius javanicus
Salminus brasiliensis
Salvelinus namaycush
Sarotherodon galilaeus
Sarotherodon melanotheron
Sciaenops ocellatus
Seriola dumerili
Seriola quinqueradiata
Seriola rivoliana
Siganus canaliculatus
Silurus asotus
Sparidentex hasta
Systomus sarana
Takifugu obscurus
Takifugu rubripes
Thunnus maccoyii
Thunnus orientalis
Thunnus thynnus
Tilapia rendalli
Tilapia zillii / Coptodon zillii
Tor tambroides
Trachinotus blochii
Trachinotus carolinus
Trachurus capensis
Trachurus japonicus
Trichogaster pectoralis
Umbrina cirrosa
Wallago attu