STUDY

Requested by the ANIT Committee

Particular welfare needs in animal transport: aquatic animals

Workshop on Animal Welfare during Transport of 25 May 2021

Policy Department for Structural and Cohesion Policies
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RESEARCH FOR ANIT COMMITTEE

Particular welfare needs in animal transport: aquatic animals

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Abstract

The purpose of this study is to analyse the particular welfare needs in live animal transport of aquatic animals. The in-depth analysis describes the key causes of suffering in relation to the needs of farmed fish, and explores strengths and weaknesses in the EU regulation and in current guidelines. Recommendations are made to mitigate the many welfare challenges identified in the study.

This document was requested by the European Parliament's Committee of Inquiry on the Protection of Animals during Transport (ANIT).

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LIST OF ABBREVIATIONS

ANIT	European Parliament's Committee of Inquiry on the Protection of Animals during Transport
EU	European Union
OIE	World Organisation for Animal Health
RAS	Recirculating Aquaculture System

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EXECUTIVE SUMMARY

KEY FINDINGS

- Fish are sentient beings and live transport inherently presents major challenges to their ability to cope with handling stressors and with their environment.
- Maintaining the best possible welfare for the fish requires careful planning, gentle loading, monitoring and maintaining conditions during the journey, gentle unloading, and monitoring in the days after unloading.
- The relevant provisions in the EU's animal transport regulation fall short of OIE standards in key areas including the allocation of responsibilities, elements of journey planning, ensuring fitness to travel, monitoring and maintaining water quality, design of vehicles and fittings, and post-transport monitoring.
- Guidelines and protocols are increasingly available across the EU. There is duplication of effort in developing guidelines, some divergence in standards, and no guideline is comprehensive. The best guidelines fall short on contingency planning for all journeys, in-depth national and sectoral guidelines address some of the critical issues, and third-party certification schemes have implemented only some relevant criteria.
- It is recommended to update EU legislation to reflect current knowledge on the needs of fish and on transport methods and to exceed OIE standards.

A diverse range of aquatic animals are transported alive in the EU, and the large portion of these movements are transports of live finfish within commercial aquaculture production. Finfish are sentient and self-aware organisms that can feel pain, distress and other emotions. Live transport of fish is inherently stressful at best and comes with significant risks of acute welfare problems. Using fish in aquaculture comes with the responsibility to safeguard and provide for the welfare of the fish, and paying high attention to fish welfare during transport is essential for the survival of the fish during transport and for reducing incidences of disease following transport.

As the knowledge base on fish welfare grows exponentially, there is a matching rise in public attention and concern for the welfare of fish. This study sets out to analyse the particular welfare needs in live animal transport of aquatic animals and to provide concrete policy recommendations on animal welfare standards for EU fisheries and aquaculture development.

Mitigating the welfare implications of transport starts with pre-transport planning and preparations. To avoid mortalities due to out of control water quality parameters (which can happen very rapidly), the transporter needs to know the size and number of fish before transport in order to load only a suitable density of fish. The vehicle needs to have the necessary equipment to monitor and maintain water quality parameters. The journey needs to be planned taking these factors into account and making assumptions for delays. It is often necessary to starve fish in the days before transport to clear their gut, reducing their metabolism and activity and reducing their excretion of waste into their transport water, and this must be carried out carefully not to create undue suffering for the fish. To cope with the rigours and stresses of transport fish need to be in good condition when they are loaded.

Loading is often the most stressful part of live fish transport. Gentle crowding, using equipment such as pumps that allow the fish to remain submerged in water, and ensuring sufficient trained personnel are available, are all important to prevent the stresses of loading from overwhelming the fish's capacity to cope. Already during loading it is necessary to provide supplemental oxygen to meet the fish's needs.

During the journey it is essential to continually monitor key water quality parameters, to supply the necessary supplemental oxygen, and to employ further techniques and equipment to manage the continually deteriorating water quality and maintain an environment in which fish can cope. Most transports use closed systems on trucks, and sometimes in boats or aeroplanes. Increasingly, well boats are used in marine aquaculture and allow for continual water exchange during the journey which provides the best water quality possible.

Unloading involves similar handling stressors to loading. Additionally, it is necessary to acclimatise fish to the water they will be unloaded into.

The stress of transport affects physiology and appetite for days after unloading. Ongoing monitoring is necessary to identify problems caused by transport, such as injuries, or an increased incidence of disease resulting from reduced immune function and/or increased exposure to infection.

Atlantic salmon and rainbow trout are especially vulnerable to poor water quality and especially to low oxygen and high CO^2 levels. They present special challenges when they are transported to sea cages as they undergo great physiological changes at this time. Gilthead seabream and European seabass are both very sensitive to the stresses of handling, and are vulnerable to rapidly deteriorating water quality which can occur in their high temperature environments and with low water exchange. Common carp is especially vulnerable to being transported with full gut and to stressors during loading. African catfish is most stressed by the transport stage and is sensitive to both high and low densities, with aggression resulting from poor density management. Turbot are normally taken out of water and kept at low temperatures to reduce the metabolism enough to cope with transport.

The World Organisation for Animal Health (OIE) adopted a chapter of baseline standards on fish welfare during transport in 2009. The EU's animal transport regulation falls short of the OIE standards in several key areas including the allocation of responsibilities, elements of journey planning, ensuring fitness to travel, monitoring and maintaining water quality, design of vehicles and fittings, and post-transport monitoring. In practice, for fish to survive live transport they require attention to their welfare that far exceeds the provisions made in EU legislation. A study of common practices during fish transport in the EU found that in most cases aquaculture operators and transporters in the EU are carrying out fish transports using procedures that meet OIE standards.

Fish transport operators in the EU require significant technical and biological expertise. They are supported by their experience and by experts in governmental agencies, research institutes, and fish health services. Support includes written standards which are increasingly emergent across Europe's aquaculture sectors. Two government authorities have produced the most detailed guidelines available in the EU, specific to their regional and national contexts. They call short of OIE standards at least in relation to contingency planning for all journeys. In other cases, national projects to improve fish welfare and fish health in aquaculture have produced less detailed best practice guidelines and standards which address several key issues but each miss key aspects of fish welfare during transport. Some third party certification schemes have also included a few criteria on fish welfare during transport.

This report presents a set of recommendations for minimising the welfare impacts of handling practices associated with transport. The design and fittings of the vehicle or vessel must be sufficient to monitor and maintain key water quality parameters throughout the journey, and have features that allow for fast and gentle loading and unloading without injuring the fish. Fish should normally have a starvation period prior to loading to prepare them for the journey, and they should be inspected so that fish with impaired welfare are not loaded. During the journey, supplementary oxygen is required and a range of equipment is necessary to continually monitor and maintain key water quality parameters including oxygen, temperature, CO^2, pH, ammonia. Loading and unloading the fish must be done gently to reduce stress and avoid injury, and the use of pumps is preferable to lifting with nets. Fish may require acclimation before being put into new water bodies, especially before unloading. The welfare impacts of transport continue for days after unloading and ongoing monitoring is necessary to identify and mitigate problems caused during transport.

1. INTRODUCTION

KEY FINDINGS

- A diverse range of animals that may be transported are included in the category 'aquatic animals'. The majority of live transports of aquatic animals in the EU are movements of finfish in commercial aquaculture, and this is the focus of this study.
- The study describes the key causes of suffering associated with transport in relation to the particular needs of farmed fish species, compares EU legislation and practice to international standards, analyses guidelines in use today, and makes policy recommendations appropriate for EU legislation.

1.1. Scope

'Aquatic animals' is a broad group of diverse animals which may be transported in Europe; especially finfish (fish), and also crustaceans, cephalopods, cetaceans, amphibians (such as frogs), reptiles (such as turtles), other marine mammals (such as seals), and other invertebrates (such as mussels). They may be transported for a range of uses; especially for commercial aquaculture production, also in other areas of the food chain, or for use in research, zoos or aquaria. Transport requires the close confinement of these animals in highly unnatural and highly controlled environments and welfare challenges are inherent.

Reviews of the literature on common practices and welfare implications in handling, storage and transport of several species of crustacean by McAnea, L. (2020a, 2020b) find that high mortality rates can be common, gentle handling is extremely important, fasting periods are commonly used as is exposure to air, and stocking densities can be too high among other welfare hazards.

Transporting cephalopods especially requires careful control of the water temperature, it is recommended to provide enrichment for them to hold during the journey, and the transport in plastic bags uses similar methods to those described for fish below (Ross, R, 2021).

The transport of marine mammals requires a range of specific measures and is addressed in a range of guidelines and protocols including those developed within the framework of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2021).

The transport of reptiles and amphibians requires attention on food, space and moisture and is addressed in a range of guidelines and protocols including the code of good practice for the husbandry of these species established by the Government of Queensland (2020).

The majority of live aquatic animal transports in Europe are fish transports in the context of commercial aquaculture, and the transport of live finfish in aquaculture is the focus of this report.

1.2. Study Methods

Chapter 2, 'Fish Welfare During Transport in Aquaculture' describes the steps and practices involved in live fish transport and the ways in which they can impact on fish welfare.

Chapter 3, 'Welfare During Transport of the EU's Major Aquaculture Species' is a literature review of the welfare and physiological needs of each of Europe's seven major aquaculture species, including how transport practices relate to these needs.

Chapter 4, 'Fish Transport in the EU and OIE Standards' compares regulatory requirements in the EU to fish welfare during transport standards in the OIE aquatic animal health code (OIE, 2019). It presents the results of a line-by-line comparison of the OIE standards and the current EU animal transport regulation (European Union, 2005), and recalls the relevant findings from the European Commission's 2017 study (IBF, et al. 2017) on fish welfare during common transport practices in the EU.

Chapter 5 'Protocols in Use in the EU' compares the fish welfare criteria included in national guidelines and in aquaculture production and sustainability standards in the EU.

Chapter 6 'Recommendations' includes policy recommendations derived from the preceding chapters, especially on operational aspects of live fish transport that are suitable for integration into EU legislation on animal welfare.

The annex 'Transport with EU Aquaculture Production Systems' uses the commercial and research experience of the authors plus literature review to describe the production contexts of live fish transports in European aquaculture.

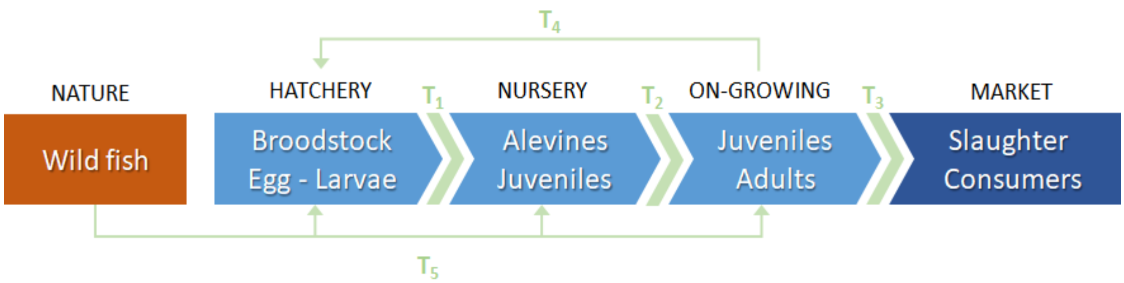
2. FISH WELFARE DURING COMMON TRANSPORT PRACTICES

KEY FINDINGS

- Finfish are sentient and self-aware organisms that can feel pain and distress, have long-term and short-term memory, and can experience emotions.
- All farmed fish will be transported at least once and may be transported as larvae, juveniles and/or as adults. Transportation of live fish involves routines that contribute to a significant increase in stress and the impairment of fish welfare.
- Inappropriate starvation periods can deplete immune function and body condition. Overcrowding can lead to poor water quality and mass mortalities as well as social distress.
- Loading is often the most stressful part of the transportation process. Gentle harvesting and handling procedures and equipment are necessary to avoid physical injuries, disruption of fishes' protective mucous coating, excessive stress and increased incidence of disease.
- Gentle movements and the maintenance of several critical water quality parameters are required for welfare and survival during the journey.
- The welfare impairments from transport continue for days after unloading.

There is strong evidence that finfish, like other vertebrate animals, are sentient and self-aware organisms, they can feel pain and distress, they have long-term and short-term memory and they can experience emotions (EFSA, 2019, Brown and Dorey 2019). Aquaculture practices frequently expose fish to a range of stressors (e.g. handling, vaccinations, crowding, grading, starvation, treatments, loading, and transportation), which do not exist for wild fish, and therefore, welfare of farmed fish must be ensured during aquaculture procedures (Conte 2004; Ashley 2007). Transportation of live fish usually involves different routines that contribute to a significant increase of stress condition and to impairment of fish welfare, and these depend on the reason for shipping, size of consignment, transport system, and species to be transported (Hastein et al. 2005). Farmed fish are often transported numerous times during their life cycle, meaning that they are exposed to various stressors during diverse transporting procedures, and these can take place between companies or sites (van de Vis et al. 2020). Fish life stages are analogous between species, regardless of the farming system, and include from eggs to adults (Fig. 1).

Figure 1: **Schematic representation of the life stages/value chain of aquaculture fish, highlighting where transportation of live fish (T) may occur**

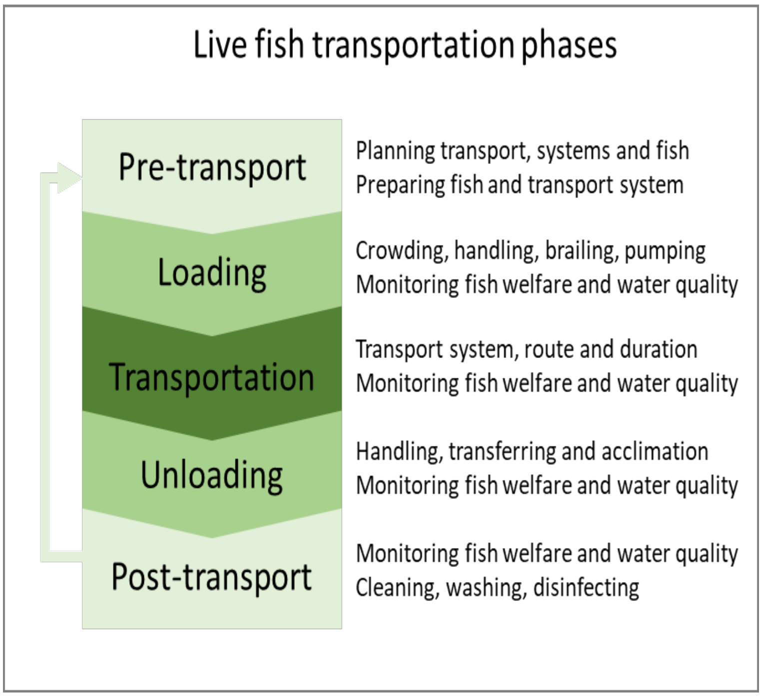


Selected broodstocks spawn in the rearing facilities where fertilized eggs are collected and raised in incubators until hatching larvae learn to swim. Then, larvae are distributed to external hatcheries or intensive fingerling tanks, which can be allocated in the same site or in other facilities, transport being necessary in the second case (T1). At this stage, larvae run out of nutritional reserves and start to feed from external sources becoming alevine. Alevine grow fast and become juvenile quickly. When they reach the correct size they are usually moved to rearing tanks of nursery areas which can be allocated in the same facility or in external ones, and transportation is again necessary (T1). They are transported again to the grow-out farming systems when they reach the appropriate size (T2), and they usually remain there until marketable size, and if not slaughtered on-farm they are transported (T3) to the slaughtering process or to be sold alive directly to the consumer. There are occasions when juveniles are reared to mature adults, and some individuals may be selected as broodstock and transferred back to breeding systems (T4). In addition, some species and in some cases wild individuals are caught and brought to captivity (T5).

Transporting live fish is a multiple-phase operation (Fig. 2). From the initial preparation of the fish, through handling and loading and then the conditions experienced during the journey itself, to the eventual unloading and acclimation to the new environment, the fish may suffer physical damage, sub-optimal environmental conditions and stress (Southgate 2008). Therefore, transporting live fish should be designed and operated to minimize or avoid stress, ensuring the welfare of farmed fish (Conte et al. 2004). The impact of transportation on fish welfare will vary according to three main interrelated sets of factors: fish, non-fish, and human factors (Southgate 2008).

Fish must be fit for transport, and stressed, diseased, or damaged fish are at high risk of acute suffering during transport. Moreover, the ability of the fish to tolerate transport routines and procedures (e.g. handling, crowding, and physical disturbance) varies with species, age, size, and physiological condition. The impact on the welfare of the transported fish will also depend on non-fish factors, such as the method of handling and loading at the beginning and end of the journey, the method of transportation, the quality of the water during transport, the stocking density, the duration of the journey, the weather, the degree of physical disturbance occurring during the journey, and the degree of biosecurity present, among others. In addition, transportation is frequently in the hands of a third party and out of the control of the supplier or recipient of the fish. It is therefore important that personnel involved in transport (human factors) are adequately trained in appropriate handling and environmental control during the journey, aware of potential effects on fish welfare, and able to identify poor welfare conditions and biosecurity risks. Based on literature reviewed (e.g. Southgate 2008; Rosten & Kristensen 2010; IBF et al. 2017; van de Vis et al. 2020), the following phases can be identified for transportation of live fish in aquaculture (Fig. 2):

Figure 2: **Live fish transportation phases and the main related welfare aspects.**



2.1. Pre-transport Planning and Preparations

Good planning is essential to be able to carry out a transport with high welfare standards. During this first phase, specific attention must be paid to planning the transport, especially to the quantity and condition of the fish, preparation of the fish prior to transport, the transport system and route, the loading and unloading procedures, the post-transport procedures, and welfare-monitoring program at each step. Planning every aspect related to live fish transportation in advance gives full control of the procedure, ensuring the best possible welfare conditions to farmed fish during transport and also ensuring mitigation and contingency plans.

It is essential to get full control of the biomass load in the fish haul, including knowing the average weight and the number of fish which represent the limit to how many fish can be loaded into a specific transport system. Overloading is a critical action in fish transport which may lead to mass mortalities due to hypoxia, accumulation of ammonia and other toxic compounds, abrupt changes in pH, temperature peaks, and overall rapid and irreversible deterioration of water quality. It may also cause social distress due to confinement and overcrowding. In addition, fish size and fitness can impact the welfare of the fish during transport (e.g. weakened fish are less likely to handle the stress a transport procedure will cause). Having information about the health status and amount of fish prior to the transport is essential to ensure good welfare conditions during transport and that proper mitigation plans can be prepared and implemented. Counting and sorting out the right sizes and healthy individuals into delivery tanks/cages allows for preparing the right number of fish for the transport and for the removal of individual fish not fit for transport. However, fish can also be directly transferred through an inline fish counting system during loading, being directly transferred towards the transport containers regardless of the system. In this case the fish owner/dispatcher and transporter carry out a quality control prior to loading fish and make sure that all necessary information about the fish group is attained and available to all relevant parties.

There are three systems for transporting live fish in aquaculture: road transporters, boats, and air freight (see below “Transportation phase”). Lorries or well-boats are the most common way of transporting fish from hatcheries or nurseries to grow-out farms, but the type of vehicle used mostly depends on whether the fish are bound for sea-water, cage-culture or inland pond-farming, as well as the distance of the route. In addition, the overall length of the transport might influence the water quality during the journey, depending on whether it is an open or a closed haul transport system, and can therefore have a direct influence on the welfare of the fish. Consequently, the distance and expected length of time of the journey are estimated and taken into account in the transporting planning within this phase.

Exposing fish to starvation for some days prior to transport is a common practice with many species. A starvation period prior to transport serves to evacuate the fish’s gut and to slow down metabolism, reducing oxygen demand and waste production during transport. Due to this mechanism, feed withdrawal before transport is a way to improve the water quality during the transport, since it is significantly affected by accumulation of potential toxic metabolites (e.g. ammonia, carbon dioxide). The reduced metabolic rate reduces activity of certain fish species and therefore also reduces stress caused by handling. Indeed, some species and life-stages (e.g. broodstocks) may be tranquilised or sedated prior to transportation. However, excessive food deprivation can result in depletion of immune status, body reserves and loss of body condition, which are associated with poor welfare.

Preparation for transport should include consideration of the fitness of the fish to be transported and the health and welfare implications for the fish being transported, but also the populations they are to join, as well as the nature and duration of the transport. In fact, transport may affect not only the individuals who are being carried, but also the animals in the destination enclosures (if any), which live in stable groups of a certain dimension, and unloading of new fish would increase the population size abruptly. Apart from inducing social stress, the new fish may also be disrupting water quality and characteristics (this may be especially relevant in ponds with limited water exchange or RAS systems).

Checking the equipment and preparation of the fish transport system prior to transportation is important for the delivery of a suitable environment for the fish. Supersaturation with gaseous nitrogen can occur during filling water into an empty haul and when starting circulation pumps, being detrimental to the health of fish and particularly problematic during transport with closed systems. Oxygenation systems and CO^2 degassing systems are to be seen as life sustaining equipment in fish haul systems, and their functionality should be tested before loading and transport. Failure to do so may result in the death of the whole population (in the cause of hypoxia or CO^2 accumulation) or severe oxidative damage in the case of hyperoxia.

2.2. Loading

The loading of fish is a crucial phase with high possibilities of having an impact on the welfare of the fish, and evidence suggests that this part of the transport process is the most stressful phase for most farmed fish species. In many aquaculture systems, the loading process begins by crowding the fish using nets and then transferring them by hand-nets, brail nets or pumping into the transport container or vehicle. The welfare impacts of loading can be reduced by various methods that allow the fish to be maintained in water, or at least reduce the time the fish are out of the water to an absolute minimum. Physical contact between fish and other surfaces, dropping fish from pumps or elevators, handling before loading, and the loading itself cause poor welfare. Crowding can be particularly stressful and it should be performed in such a way that fish do not show signs of distress. Pumping and poor handling may result in physical injuries, particularly to the fins, but also disrupting the protective mucous coating and fish scales, thereby increasing susceptibility to parasitic or pathogenic invasion. Moreover, handling and netting fish usually requires imposing an intrusive foreign element into the water to corral and catch the fish, and if not done correctly excessive stress can jeopardize fish welfare. All fish-handling processes should be slow and deliberate so as not to increase the natural avoidance reactions of fish, which can lead to excessive activity, stress, and potential exhaustion. Excessive gravitational weight loading on fish positioned at the bottom of the lift net can cause injuries from compression and spine injuries from adjacent fish. Damage to the fish might also occur during pumping into containers if water is lacking or if speed and g-force is too high, causing fish to hit walls, misfitting joints, or sharp edges in the transport hose/pipe. Fish escapes might also occur during loading, particularly when transferring into/from well-boats, leading to welfare impairments on escapees and environmental problems.

Exceeding the capacity of the vessel in terms of fish load and fish density can have negative effects on the welfare of fish in transport. Biomass estimating equipment is available and applied in many large well-boats, allowing calculation of the size, distribution and numbers of fish brought into the hull through mathematical models derived from empirical datasets. In addition, a sufficiently large number of skilled people is necessary to ensure that the loading process runs as smoothly as possible, ensuring welfare of transferred fish. Coordination between fish owner and transporter is essential to avoid too long crowding operations before and during loading, as well as other operational related problems that may cause impairments in fish welfare.

Oxygen is the most critical parameter to control in aquaculture, both too little (hypoxia) and too high (hyperoxia) can create welfare problems for fish. However, oxygen supply and CO^2 degassing can partly counteract this. Both high and low oxygen levels are particularly dangerous in combination with high levels of metabolites in the transport water. For example, the toxicity of ammonia increases with low oxygen levels, and hyperoxic water causes increased internal oxygen concentrations, which might cause oxidative damage (i.e., loss of cell functioning due to accumulation of toxic free radicals) and have negative effects on osmoregulation, which can lead to salt and water imbalances in the organism. All these events can be fatal if not controlled and reversed in a short time. The water in the transportation unit should not contain high levels of metabolites, metals or organic matter and should be controlled with biosecurity in mind.

2.3. Transportation

The transport phase consists of moving fish from the sender to the receiver and it can be challenging and unpredictable. As mentioned above, there are three main systems for transporting live fish in aquaculture: road transporters, well-boats, and air freight. Road transport of farmed fish is frequently used for transferring fish from a hatchery to an on-growing facility, and is usually carried out in multiple purpose-built tanks on a road haulage vehicle. These tanks are usually constructed of fibreglass with a sealable loading hatch and a valve/pipe discharge point. Oxygen is supplied from on-board cylinders and dissolved oxygen and temperature in water is continuously monitored from within the cab of the vehicle. Single tanks with oxygen supply can be used for small numbers of fish, relevant specimens, or short-distance transportations (e.g. broodstocks). Well-boats are becoming increasingly used in marine fish farming, both for transporting juvenile fish to on-growing sites and for moving harvest-sized fish to central slaughter stations. Fish are pumped from road transporters or sea-cages into chambers (wells) in the hull of the boat, oxygen is provided and water is circulated through these chambers, either as an open system with water being constantly replenished through open valves or, in some circumstances, with closed valves giving 100% recirculation (due to biosecurity or the requirement to chill the fish). Indeed, deciding whether and when the transport is carried out as a closed or open haul transport is crucial. Nevertheless, a variety of water quality parameters are monitored and most well-boats are equipped with continuous video monitoring of the fish.

Much less common is the use of towing sea-cages with fish by boat, where cages of market-sized fish are towed to a land-based processing plant where the fish are pumped out, stunned and slaughtered. In capture-based aquaculture, tuna is transferred by towing sea-cages from purse-seine vessels to on-growing cages. Transport of live fish by air is not commonly used in aquaculture, with two exceptions. The first one is transporting Atlantic salmon smolts from land to on-growing sites at sea in a relatively short-time operation. In this specific case, fish are loaded from road transporters into tanks hanging beneath helicopters, and these tanks are then sealed and flown to the sea-pens, where the tank is lowered into the water within the pen and a float mechanism on the tank releases the fish into the water. The second one is when fish have to be transported for very long distances (between distant countries, within large countries, or across continents), then air transport is used for fry or fingerlings. Here, fish are placed in plastic bags and then bags are inflated by oxygen and packaged in insulated polystyrene boxes for air transport. Regardless of the transport system, contemporary long distance containers are insulated and equipped with chillers, carbon dioxide strippers, anti-foam agents, water buffers, circulation pumps, and oxygen sources. Short-haul tank trucks are usually equipped with ice, circulation pumps, and anti-foam agents.

During transport the principal concern is for maintenance of satisfactory water quality (e.g. oxygen, carbon dioxide and ammonia levels, pH, temperature, and salinity) appropriate to the species being transported. Deterioration of water quality during transport is the most significant animal welfare issue for transporting live fish, especially the depletion of oxygen or accumulation of carbon dioxide and ammonia. Deciding whether and when the transport is carried out as a closed or open haul transport is of critical importance to water quality. Transport of live fish in a well-boat gives the choice of carrying out the transport using either an open single-pass flow-through system or a closed haul system, whereas in road transportation (and air freight) the only available option is using a closed system. When transporting in well-boats, selecting an open or a closed system depends upon whether there are bio-security issues associated with the fish group itself, local regulations (e.g., coming into a slaughter facility) or risks of contamination with fish pathogens along the transport route. Bio-security viable open transport is preferable in terms of water quality in the fish haul, given that a single-pass flow-through system will provide a substantial amount of the necessary oxygen for the fish and will have a higher capacity to remove metabolites, such as CO^2 and ammonia compounds, which might cause welfare problems. However, a single-pass flow-through system can compromise the welfare of fish if the route passes through polluted areas or areas contaminated with parasites (i.e., contamination passing from the wild to transported fish) or can cause environmental problems if the transported fish are infected (i.e., contamination passing from transported fish to the wild).

2.4. Unloading

Unloading can be a critical phase and fish should be unloaded as soon as possible, given that similar to the loading phase, fish stress increases significantly during this phase.

Unloading manoeuvres are diverse, but pumping fish and water through a pipe/hose into the new tank or net pen is the most common and extended practice, and fish are never exposed to air. In order to unload the fish through pumps, pressure can be increased (about 0.1 - 0.2 atm inside the haul by adding water or lifting height) and such pressure difference drives the fish and water together through the pipe. However, gravity, vacuum pumps, or water filled dip nets can be used when lifting to greater heights. Brailing with nets are also used for unloading at a small-scale or with a few individuals, and in this case air-exposure has to be kept to the absolute minimum. Similar to loading, physical contact between fish and other surfaces from pumps or nets, and handling and dropping fish from pumps or elevators, cause poor welfare. Pumping and poor handling may result in physical injuries, particularly to the fins. Damages to the fish might also occur during unloading with pumps if water speed and g-force is too high, causing fish to hit walls, or misfitting joints, or sharp edges in the transport hose/pipe.

2.5. Post-transport

This phase is highly relevant to ensure good welfare conditions of transported fish and to identify possible problems and solutions. It can also potentially affect the welfare of the next fish group in the transport. The post-transport phase includes monitoring fish welfare, identifying any damage or poor condition, as well as losses due to any aspect of transport, including loading and unloading, and any increased incidence of disease in the days after unloading. After delivery, the haul and circulation systems are cleaned and washed after unloading, removing all organic material, followed by disinfection. Removal or disinfection of transport water is also considered in this phase.

3. WELFARE CONCERNS DURING TRANSPORT OF THE EU'S MAJOR AQUACULTURE SPECIES

KEY FINDINGS

- **Atlantic salmon**. Extra challenges exist when transport is of smolt (juveniles) from freshwater to sea water. Excessive starvations periods increase aggression and fin damage. Sensitive to accumulation of CO^2.
- **Rainbow trout**. Acute or repeated stress in juvenile stages negatively affects physiology, behaviour and growth. Demonstrate high levels of activity and stress during transport and require a recovery period following transport.
- **Gilthead seabream**. Show severe signs of stress when crowded, exposed to air or confined. Water quality deteriorates significantly when water exchange is low and temperatures are high.
- **European seabass**. Juveniles are especially sensitive to handling stressors inherent in transport, and to short-term exposure to poor water quality.
- **Common carp**. Appropriate starvation and then reducing stress during loading are particularly important.
- **African catfish**. Experiences the most stress during transport itself and sensitive both to too high densities and too low densities.
- **Turbot**. Normally require low temperatures and transport in air to reduce metabolism enough to undergo live transport.

The effects of the different stressors related to live fish transportation greatly depend on the transport system and equipment, but also on the fish's capacity to cope with stressful conditions, the biological requirements of each species, as well as on the developmental stage. The most relevant aspects related to the welfare during live transportation of the seven most produced species in European aquaculture are detailed below. The annex 'Transport Within EU Aquaculture Production Systems' explains the aquaculture production and transport systems that determine the timing and nature of live transports of the seven most produced species in European aquaculture.

3.1. Atlantic Salmon (*Salmo Salar*)

The initial procedures of live fish transportation involved in handling smolts, such as gathering fish together, counting, capturing and loading are the most stressful stages of salmon transport, but also crowding, pumping, vaccination and sorting during the freshwater phase also expose the smolts to welfare challenges. Moreover, several potential stressors are also involved during road, sea and air transport of smolts and adults, including handling and loading, alterations in water quality, osmoregularity disruption and novel transport containers (Santurntun et al. 2018). Some studies indicate that salmon fry can be stressed by confinement and handling (Barton 2000; Vaz-Serrano et al. 2011), as well as salmon parr and adults that get highly stressed by acute handling, having direct consequences on feed consumption and growth (McCormick et al. 1998). Indeed, during the pre-transport phase fish might be handled more frequently, having more rapid and greater effect on salmon stress (McCormick et al. 1998). Stressors such as chasing, netting or emptying the tank also have a strong effect on hypothalamic-pituitary gland–interrenal gland (HPI) axis as a result of chronic stress in Atlantic salmon juveniles (Madaro et al. 2015). Cortisol elevation is known to occur in salmon kept out of water in a net for a short period and can be associated with immunosuppression (Maule & Schreck 1991), and such physiological effects of being captured can remain even after 48 h (Iversen et al 1998).

When transporting salmon smolts to sea-cages by well boats, the loading process is a more severe stressor than the transport itself (Iversen et al. 2005). The stress of transfer from fresh- to seawater can be ameliorated by a sedative, such as isoeugenol (Iversen et al. 2009) but the full impact of this on welfare remains to be determined. Plasma cortisol increases were observed during loading smolts to the well boat through pumps, and also during the loading phase, whereas cortisol returned to resting levels during transport. This suggests that well boats can provide an important recovery function. However, unfavourable weather conditions at sea may prevent such recovery during the live haul transport trip, and inappropriate water quality conditions (e.g. O2, CO^2, pH, temperature) and monitoring/life-supporting systems might provoke serious impairments of salmon welfare and flesh quality (e.g. Tang et al. 2009, Farrel et al. 2010). Salmonids are sensitive to accumulation of expired CO2 during transport, and similar argument can be applied to road transport (King 2009). Consistent with previous findings, another study indicates that the greatest stress occurs during truck transport and loading (Nomura et al. 2009). Salmon smolts showed physiological stress when loaded and transported from freshwater tanks via tanker truck to a dock (fish density = 70 kg/m3) and then loaded in to the holds of the waiting live-haul vessel before being moved to sea pens. The primary and secondary stress responses initially observed as the fish were first transferred became progressively resolved during the period of time that the fish were held at dockside (after a period of 4-h loading), but also during the 4-h sea transport (fish density = 20-30 kg/m3) (Nomura et al. 2009). It must be noticed that smolts may accumulate at the bottom of the well-boat tanks as an aggregation response to the stress of the loading procedure (Nomura et al 2009), and that cortisol concentrations can remain high for 1–2 days after transport (Barton 2000). Similarly, a study on salmon adults also indicates that one of the most stressful stages during transportation is the pumping from the resting cages to the processing plant, which confirms that the latter handling is the most stressful of the stages studied during commercial transport (Gatica et al. 2010).

Prior to any transportation and slaughter, Atlantic salmon are often deprived of food for some days or weeks. Restricting feed to salmon for up to 10 days can decrease weight, length and fish body condition, but also increase fin erosion due to increased aggressiveness (Jones et al. 2017). Crowding before slaughter leads to increased levels of cortisol, lactate and osmolality in blood plasma of adults salmon, indicating significant pre-slaughter stress, and also provoking negative effects on fillet quality (Skjervold et al. 2001). It must be noticed that many of the disease outbreaks take place during the first months of transfer to the sea following transport in well boats (Iversen et al. 2005). Recovery after road or sea transport was reported to reduce blood stress measures and improve fish survival (Finstad et al 2003; Gatica et al. 2010).

3.2. Rainbow Trout (*Oncorhynchus Mykiss*)

Similar to Atlantic salmon, there are several procedures during transportation that can expose rainbow trout to stress and welfare impairments in every life-stage of the production cycle. For example, many studies have previously reported that acute or repeated handling, netting, crowding and confinement negatively affect physiology, behaviour and growth performance of rainbow trout alevines (Barry et al.

1995; Sadoul et al. 2015), smolts (Vijayan and Moon. 1992; Kubilay and Ulukoy 2002; Ellis et al. 2004; Jentoft et al. 2005; Hoskonen and Pirhonen 2006; Pottinger 2006; Laursen et al. 2013), adults (Pickering et al. 1991; Sloman et al. 2001; Lepage et al. 2002; Øverli et al. 2006; Pottinger 2006) and breeders (Wagner et al. 2002; Rexroad et al. 2012; Weber et al. 2008). Live transport, including loading and unloading, represents a major stressor for rainbow trout (Shabani et al. 2016). Indeed, regular loading and unloading operations, such as crowding and pumping, can cause higher stress on rainbow trout than the journey, and have direct negative effects on flesh quality accelerating the onset of rigor mortis in sea-farmed rainbow trout (Merkin et al. 2010). During road transport by truck, heavy oxygen supersaturation and elevated levels of CO^2 can be detected in transport water as a consequence of excessive respiration rates and stressful conditions (including suboptimal water quality) (Shabani et al. 2016). Another study indicates that rainbow trout show vigorous swimming activity and elevated oxygen consumption during road transport by commercial truck (Chandroo et al. 2005). While activity levels returned to baseline within 48 h, beyond this period, swimming performance, measured as critical speed and endurance, was still affected. A similar study demonstrated that during handling and immediately after road transport rainbow trout smolts showed high levels of plasma cortisol, even higher levels three hours after transport, and were decreasing during time without reaching basal levels within the following 48 hours (Barton et al. 2000). Therefore, the provision of a recovery period following transport is clearly important for welfare and subsequent survival.

In addition, rainbow trout presents a net loss of sodium and chloride ions associated with the stress of transport, so they benefit from dilute salt solutions or a current during, before or after transportation, ameliorating in turn the skin condition and microbiota (Pickering, 1992; Tacchi et al. 2015). Moreover, inappropriate water quality conditions (e.g. O2, CO^2, pH, temperature) and monitoring/life-supporting systems can lead to welfare problems for rainbow trout (MacIntyre et al. 2008). Similar to Atlantic salmon, gradual cooling is considered a primary non-chemical technique to reduce stress of rainbow trout during transportation, but the temperature should not be below 6ºC (Coyle et al. 2004; IBF et al. 2017). Prior to any transportation and slaughter, rainbow trout are often deprived of food for some days or weeks, which may benefit welfare by reducing metabolism, oxygen demand and waste production during live fish transportation. Whereas long fasting periods can be highly detrimental for the fish welfare (Kakizawa et al. 1995; Furné et al. 2009), feed deprivation for short periods under appropriate conditions may not diminish welfare. However, reduced food abundance and accessibility can cause changes in territorial behaviour strategies and social hierarchies in rainbow trout (Boujard et al. 2002).

3.3. Gilthead Seabream (*Sparus Aurata*) and European Seabass (*Dicentrarchus Labrax*)

The pre-transport procedures may be more stressful for seabream than the journey. For example, larvae and young are stressed by air exposure (Van Anholt et al., 2004) and handling (Alves Martins et al., 2012; Koven et al., 2001; Van Anholt et al., 2004). Both juveniles and adult fish show severe signs of stress when crowded, exposed to air and confined (Arends et al., 1999; Bagni et al., 2007; Montero et al., 1999; Rotllant et al., 2001; Tort et al., 1996). Noise from transport operations may also be a factor in stress (Filiciotto et al., 2013). Water quality may also deteriorate during transport, especially due to low water exchange and high temperatures. Oxygen below 70% (Araújo-Luna et al., 2018) may cause hypoxia, and ammonia above 1.5 mg/L and temperature above 28ºC may cause severe welfare impairments after 30 minutes (Zarantoniello et al., 2021) or death if prolonged. Oxygen must be supplemented and monitored continuously during transport, and ammonia and CO^2 should also be monitored. Regarding noise, juvenile and adult gilthead seabream are stressed by acoustic stimuli at high frequencies and intensities.

European seabass is a delicate species in terms of stress, as mass mortalities have been reported at several fish farms and may occur during transport. Overall, European seabass alevins are very sensitive to external stressors at very early stages. European seabass juveniles and adults are stressed by handling, confinement, crowding, transport, low temperatures, and high stocking densities, but stress decreases with good maintenance conditions. Transport can also cause stress and impair welfare through crowding, handling, water movement, noise and vibrations, and poor water quality. Even a short term (5-minute) exposure to oxygen below 70%, ammonia above 1.5 mg/L or temperature above 28ºC already causes severe welfare impairments (Alfonso et al., 2020) or death if further prolonged. Ammonia can peak abruptly in transport events, as excretion rates can be 4x higher than in normal conditions. Oxygen must be supplemented and monitored continuously during transport, and ammonia and CO^2 should also be monitored. In broodstock, transport procedures also impair reproduction for a long period (EFSA 2008).

For both seabass and seabream, preparation and transport events can cause stress and impair welfare through changes in feeding, stocking density, handling, water movement, noise and vibrations, and poor water quality. Although the stressors and impacts are similar, the thresholds of stress and magnitude of impacts may differ among these species. The crowding procedure may cause mechanical damage from spines and increase the risk of infectious diseases from injury and pathogen transmission from direct contact. This risk increases exponentially if the fish are moved by dry brailing, but may be reduced if wet brailing or pumps are used. Upon loading and unloading, mechanical shock may cause internal and external injuries from contact with water surface, tank, transport or handling structures or other animals. Upon arrival, welfare hazards include differences in water parameters (namely temperature, salinity and pH) between transport and destination water. The lack of appropriate acclimation procedures may also raise welfare issues: for example, transported fish should be given enough recovery time after arrival without handling; feeding should be resumed only when the animals demonstrate appetite. If in RAS, the biological filter should also be mature to receive the planned organic load.

3.4. Common Carp (*Cyprinus Carpio*)

The first measure to consider when planning the transportation of common carp is feed deprivation, as transporting common carp with heavily filled digestive tracts can be lethal (Svobodová et al., 1999) and feeding history influences the onset of the stress response in common carp (Ruane et al., 2002). The next consideration is reducing the stress induced by the pre-transportation manipulation (crowding, netting, catching, hauling, and reloading), which is a major cause of stress for common carp with regards to transportation (Svobodová et al., 1999; Dobšíková et al., 2006, 2009). Netting can cause damage by abrasion, and there are other potential dangers that can cause pain such as hits and impacts during net chasing or during unloading ("discharging") fish from their home tanks (EFSA, 2009). Additionally, because common carps live in muddy waters that create a dim environment and the transportation manipulation usually takes place during daylight, the sudden light change is an additional negative stressful stimulus (EFSA, 2009). When transferring fish from their home enclosure to the transport containers, the water temperature must be the same in both environments as a temperature difference is a potent stressor (Svobodová et al., 1999; Küçükgül, 2008; EFSA, 2009). They are sensitive to urban noise (Kusku et al., 2018), sudden noises, vibration and high stocking densities (EFSA, 2009). Thus, the transportation per se is also a mild to severe inducer of stress (Svobodová et al., 1999; Dobšíková et al., 2006, 2009; Varga et al., 2014).

3.5. African Catfish (*Clarias Gariepinus*)

African catfish are remarkably resilient and, as air-breathing fish, can stay alive for many days outside the water as long as their skin remains wet. These characteristics sometimes incite to overlook their welfare needs. However, African catfish are sensitive to stress. For example, larvae can die after handling (Haylor, 1992). Adults are stressed when they are at very low stocking densities (500 fish/m3) because the levels of aggression are higher at these densities. They are also stressed at very high stocking densities (3000 fish/m3), probably due to crowding stress (van de Nieuwegiessen et al., 2008). Increased hours of light also induce stress and aggression in catfish (Almazan-Rueda et al., 2005). Because this species is very aggressive, the fish from production ponds must be graded to prevent cannibalistic events (Basharat et al., 2020), with medium- and heavy weight fish benefiting from living in homogeneous groups and low-weight fish benefiting from living in heterogeneous ones (Martins 2006). Transportation is also a strong stressor for both adults (Adeyemo et al., 2009; Manuel et al., 2014) and fingerling (Nasrullah et al., 2021) catfish. During the transportation event, stress starts during the fasting period, increases with handling, and gets even higher during transportation (Manuel et al., 2014). Additionally, handling and transportation induce skin lesions (Manuel et al., 2014) and have a negative effect in the immune system (Adeyemo et al., 2009; Nasrullah et al., 2021).

3.6. Turbot (*Scophthalmus Maximus*)

Similar to previously reported species, the initial procedures of live fish transportation are highly stressful for turbot. However, there is very little information on the effects of the procedures carried out at different stages of transport on the welfare of fish. Some studies reported that juvenile turbot can be stressed by handling and net confinement (Waring et al. 1996, 1997), and crowding at high densities (Jia et al. 2016). Particular attention must be paid on turbot skin, which is very sensitive, presents no scales, and exerts multiple vital protective functions against environmental aggressions (Faílde et al. 2014). Indeed, there is a cumulative effect of multiple handling stress on physiological stress in turbot, which is reduced by a 24 h recovery period between multiple handling procedures (Waring et al. 1997). Enforced exercise can also induce a moderate stress response in juvenile turbot (Van Ham et al. 2003). One common practice to reduce stress before and during transportation is exposing fish to starvation for some days. However, feed restriction promotes competition in turbot, especially at high stocking density (Martines-Tapia et al. 1991; Sunde et al. 1998; Irwing et al. 1999; Sæther and Jobling 1999). Juvenile turbot are also sensitive to salinity and temperature changes (Gaumet et al. 1994; Staurnes 1994), that can occur during transportation phases. Cold acclimation of the fish (water temperature: 2ºC, for 2 days), followed by waterless transportation with high oxygenation at low temperatures, is used to reduce the problems associated with transport of live turbot. (Nie et al. 2018, 2019, 2020). Low temperature and oxygen availability can reduce respiration and metabolism keeping fish in a dormant state and decreasing the oxygen consumption, and sustain biological homeostasis of turbot in response to water-less conditions, improving therefore the chances of survival. However, besides the physiological changes mainly associated to metabolism, hormone, innate immune system and liver function, the cold acclimation also caused adverse effect to the innate immune system and liver of turbot. In addition, one of the causes for the death of turbot during transportation is the ineffective excretion of metabolites resulting in a toxic accumulation of harmful substances like ammonia or nitrogen (Brown et al. 1984, Ruyet et al. 2003). It was shown that pH of transport water is an important factor in determining the cumulative mortality rate of turbot kept at high densities without water exchange, because of the great influence of water acidity on ammonium toxicity (Grøttum et al. 1997). Using pure oxygen instead of aeration as oxygen supply and adding acid can reduced the pH, reducing ammonia toxicity but increasing CO^2 levels.

4. OIE STANDARDS AND FISH TRANSPORT IN THE EU

The OIE publishes and maintains the OIE Aquatic Animal Health Code (OIE, 2019), including a specific chapter of standards for the welfare of farmed fish during transport. The chapter was first adopted in 2009 and last updated in 2012. The welfare chapters of the Code are now among the chapters to have gone the longest period since being updated to reflect current knowledge. All EU Member States are among the 182 members of the OIE and committed to implementing the standards, and in most cases EU regulations exceed OIE standards for the welfare of terrestrial animals.

The OIE standards are a global baseline standard against which EU regulations and practices can be benchmarked. This chapter assesses any shortcomings in the EU in the achievement of the OIE standards for fish welfare during transport.

This chapter presents the results of a line-by-line comparison of the OIE standards and the EU's animal transport regulation (European Union, 2005), and identifies shortcomings across a range of key criteria.

Shortcomings in commercial practice identified in the European Commission study 'Welfare of farmed fish: Common practices during transport and at slaughter' (IBF et al. 2017) are also presented. The study benchmarked common practices in thirteen European aquaculture sectors and found that OIE standards are met in most cases, with contingency planning absent in two sectors and water quality monitoring absent in one.

This chapter follows the structure of the relevant OIE aquatic code chapter and addresses only those aspects of the OIE chapter where specific shortfalls are identified in the EU.

4.1. Responsibilities

The OIE code establishes that the Competent Authority is responsible for 'establishing minimum standards for fish welfare during transport including examination before, during and after their transport, appropriate certification, record keeping, awareness and training of personnel involved' and for 'ensuring implementation of the standards'. The EU's animal transport regulation establishes only general standards for the welfare of farmed fish and specific shortcomings are identified below.

The OIE code establishes a responsibility for transporters to cooperate with farm owners/managers in the planning of transport including on vehicle selection, on arranging sufficient competent staff and suitable equipment for loading and unloading, and on establishing contingency plans. The EU's animal transport regulation establishes a range of responsibilities on transport and farm operators to report journey information to the competent authority, the limited requirement for the transporter to collect these reports after completion of the journey, and a general requirement on transport organisers to ensure sufficient coordination of different parts of the journey. The EU's animal transport regulation doesn't provide for the specific communication between operators that is necessary in live fish transport.

4.2. Planning

The OIE code establishes that vehicles or containers should have adequate circulation of water and equipment for oxygenation, and that equipment to monitor and maintain water quality may be required depending on the length of the journey. The EU's animal transport regulation does not address monitoring or maintaining water quality parameters.

IBF et al. (2017) identified the transport of common carp to slaughter taking place in one sector without the monitoring of any water quality parameters.

The OIE code establishes that feed should be withheld from the fish while taking into consideration the fish species and life stage, in preparation for transport. The EU's animal transport regulation recognises that some species require acclimation prior to loading, but doesn't address any other aspect of preparation of animals in the days prior to transport, such as starvation periods.

The OIE code establishes that fish should be considered unfit for transport if they show abnormal behaviour, such as rapid ventilation or abnormal swimming. The EU's animal transport regulation defines being unable to move independently without pain as the only behavioural indicator of fitness for transport.

The OIE code establishes that contingency plans are necessary for all journeys for managing and acting on adverse fish welfare events. The EU's animal transport regulation doesn't require contingency plans to be in place for journeys under eight hours in duration.

IBF et al. (2017) identified the transport of marketable common carp in two sectors taking place without contingency plans.

The OIE code establishes that the biomass load and expected time of arrival and unloading are required elements of documentation. The EU's animal transport regulation includes no requirements on these.

4.3. Loading

The OIE code establishes that it is necessary to address crowding procedures prior to loading to avoid injury and unnecessary stress. The EU's animal transport regulation makes no provision for this aspect of loading.

The OIE code establishes that equipment should be properly constructed to avoid injury and stress. The EU's animal transport regulation has the requirement that the surfaces of facilities for loading and unloading 'shall not be slippery', a requirement which must be dismissed in the case of fish loading and unloading equipment in order to avoid injury to the fish.

The OIE code establishes that the density of the fish in a vehicle or container should not exceed what is generally accepted for a given species. The EU's animal transport regulation considers only floor space and head room, and makes no provision for the three dimensional movements and environment of fish in water.

4.4. Transporting

The OIE code establishes that water quality should be monitored and adjusted. The EU's animal transport regulation doesn't address water quality parameters and has no requirements for the continual monitoring of environmental conditions.

4.5. Unloading

The OIE code establishes that moribund or seriously injured fish should be removed and humanely killed. The EU's animal transport regulation requires emergency killing when there is no other way to safeguard the welfare of the animals, but has no requirements on the removal or handling of sick or seriously injured fish.

The OIE code establishes that equipment should be properly constructed to avoid injury and stress. The EU's animal transport regulation has the requirement that the surfaces of facilities for loading and unloading 'shall not be slippery', a requirement which must be dismissed in the case of fish loading and unloading equipment in order to avoid injury to the fish.

4.6. Post-transport activities

The OIE code establishes that the person in charge of receiving the fish should closely observe them and keep appropriate records that fish showing abnormal clinical signs should be killed, isolated or examined by a veterinarian, and problems with transport should be evaluated. The EU's animal transport regulation doesn't make any provisions for the post-transport phase.

4.7. Conclusions

Live transport is inherently challenging to the welfare of fish and the commercial imperative to keep fish alive and with a good appetite and immune function has driven common practices in commercial aquaculture to exceed the minimal provisions made in EU legislation.

Many aspects of fish transport require communication and cooperation between farmers and operators before, during and after the transport. This is especially true of preparing fresh water for the vehicle, determining temperatures and the related density, oxygen provision, journey planning, and preparing for acclimation and unloading at the destination. EU regulations fail to provide for this essential communication between operators. Further, EU regulations fail to establish responsibilities for vehicles to be suitably designed and fitted to monitor and maintain water quality and to avoid stress and injury.

The preparation of fish especially the use of starvation periods is not required or controlled by the EU regulation. This process is critical to slowing the metabolism of the fish and preserving water quality during transport, and must be carried out with care to avoid causing suffering through excessive starvation periods. Further, the EU regulation allows for fish with impaired welfare and that may be unable to cope with the challenges of transport to be loaded.

Water quality can deteriorate rapidly and exponentially, and OIE standards require contingency planning for all live fish transports. The EU regulation requires this only for journeys over eight hours in length and some live transports in the EU take place without any contingency planning.

The EU regulation addresses specifics of loading equipment and requirements for terrestrial animals and without including the special requirements of fish, it extends the requirements for terrestrial animals (such as prohibiting slippery surfaces) to fish even though they may be irrelevant or inappropriate for fish.

Monitoring and maintaining key water quality parameters is perhaps the most critical aspect in the live transport of fish, and is not addressed in the EU regulation. Occasionally commercial live fish transports do not monitor any water quality parameters.

Fish are affected by live transport for several days after unloading. Monitoring their feeding, behaviour and health during this period is essential for identifying and mitigating problems caused during transport, and is not addressed in the EU regulation.

5. FISH WELFARE IN EU TRANSPORT GUIDELINES

KEY FINDINGS

- Governmental agencies, research institutes, fish health experts and experienced operators support fish transport standards including through written standards.
- Detailed guidelines have been developed by two EU Member States, criteria have been included in other fish welfare and fish health handbooks, and limited criteria are included in third party certification schemes.
- There is duplication of effort and divergence in standards as guidelines are increasingly formalised and developed.

In every case, significant technical and biological expertise is required for the live transport of fish to be successful. Operators typically have significant formal and informal knowledge gained through training and through experience. They are frequently supported by professional veterinarians, by standards and protocols produced by fish welfare experts and by producer organisations, and sometimes by guidelines produced by governmental and intergovernmental organisations.

Among the EU's Member States, Germany and Italy have guidelines in place on welfare during the live transport of fish (European Commission, 2021).

This chapter presents a summary and comparison of six fish transport guidelines and protocols used in the EU. The summaries contain specific details where they are of particular relevance to the findings and recommendations made in this report.

Governmental institutions have produced guidelines on fish welfare during transport in the German state of Bavaria (LfL, 2018), and in Italy (Ministero della Salute, 2018), that aim at imparting best practice and at achieving the requirements of the EU's animal transport regulation (European Union, 2005). They each go far beyond the OIE standards in their level of detail and in defining key parameters, each containing species-specific and in depth information and contextual details Neither addresses every element of the OIE standards (OIE, 2019) and especially they fall short in terms of contingency planning for all journeys.

Two further guidelines from two of Europe's most developed aquaculture sectors are also compared below. Fish welfare guidelines produced in Greece (the EU's largest producer of farmed fish), by academic experts in collaboration with producers and national policymakers, include transport standards (Pavlidis & Samaras, 2019). A government project in Ireland (the EU's largest producer of salmon and of organic fish) to enhance fish health and sustainability produced a fish health handbook (Marine Institute, 2011) which includes standards for fish welfare during live transport. They each contain important but more general information and guidelines with some species-specific details and some key aspects of fish welfare during transport are not addressed.

Third-party certification schemes providing for safe and sustainable aquaculture products are common in the supply chain and prominent on consumer facing packing in the EU. Two schemes already containing criteria for fish welfare during transport are compared in order to examine the role that third-party schemes have played in driving commercial standards. The GlobalGAP (2019) standard is a private certification scheme for sustainability and food safety with a high market coverage of EU aquaculture production, and the Naturland (2020) organic aquaculture standard attracts a high premium in the EU market. Several of the major schemes have current projects developing fish welfare criteria for future versions of their standards.

5.1. Authority Guidelines

Table 1: Fish welfare during transport guidelines from government agencies in the EU

Bavaria	Italy
Pre-transport	
- Presents the **vehicle licensing** regime. - Collates and presents **training requirements** from relevant regulatory frameworks. - Presents legal requirement to have **contingency plan** for long journeys. - Makes recommendations on **disinfection** - Describes different **transport systems**, necessary **equipment** and makes recommendations on best use of equipment. - Gives species-specific **starvation periods**. - Presents **documentation** and reporting requirements from relevant regulatory frameworks (pre- and post-transport).	- Gives a detailed **division of responsibilities** between operators and authorities on fish health measures, vehicle authorisation, transport planning, preparations and procedures, inspections and documentation and reporting. - Gives detail of the fish welfare aspects to be included in the necessary **training**. - Gives detail on route and **journey planning**, on **contingency planning** (including model plans), on planning on basis of 50% extra journey duration (including carrying extra oxygen) and on **disinfection** routines. - Describes different **transport systems**, necessary **equipment** and makes recommendations on best use of equipment. - Gives species-specific **starvation periods**. - Presents **documentation** and reporting requirements from relevant regulatory frameworks (pre- and post-transport).
Loading and unloading	
- Makes detailed recommendations on **gentle crowding and handling** procedures and on **acclimation**. - Gives species specific **densities** especially in relation to life stage and oxygen requirements	- Makes detailed recommendations on **gentle crowding and handling** procedures, and on planning and monitoring **density**, and on **acclimatisation**. - Gives **species-specific densities** in ranges and for different water temperatures. - Gives the legal definition of being **fit to travel**.

During the journey	
- Gives key water quality parameters, and **describes critical interactions** between **water quality parameters and fishes' needs.** - Gives **species-specific water quality parameters** in limited and in critical ranges. - Gives specific recommendations on **reducing stress** caused during the journey especially by movement of the vehicle.	- Gives key water quality parameters, and **describes critical interactions** between **water quality parameters and fishes' needs**. - Gives **species-specific temperature and oxygen parameters** for the 7 most important species, and many more parameters for the most important species (trout). - Recommends that **temperature** is measured by installed sensors and **oxygen measured every 2 to 4 hours**. - Presents the legal requirements to maintain **biosecurity when exchanging water**, and set **upper limit for journey duration** without exchange of water. - Gives specific recommendations on **reducing stress** caused during the journey especially by movement of the vehicle.
Post-transport	
-	-

5.2. Aquaculture Sector Guidelines

Table 2: Two national sectorial handbooks on fish welfare and fish health

Greece	Ireland
Pre-transport	
-	- Makes the recommendations that **staff are trained** in specific tasks and in good fish handling techniques. - Makes the recommendation that a **risk assessment** is carried out. - Makes the recommendations that **equipment is designed to minimise damage** to fish and is maintained in good working order, and has schedules for hygienic use and **disinfection**. - Gives a species-specific **starvation period**. - Presents the requirement for a **pre-loading notification** to the competent authority and

	for a **health check** on the day of loading, and presents the requirement not to load if there are health issues on the farm. - Makes a general requirement for **record keeping** to allow traceability.
Loading and unloading	
- Describes **handling** aspects in detail including with reference to transport. - **Behaviour, injuries, respiratory rate, oxygen, temperature, density, pH, and glucose, lactic acid and blood cortisol** are identified as the relevant **indicators** and described. (all stages) - Makes the general recommendation to use **best handling practices and modern equipment**.	- Gives detailed recommendations on **best practices for gentle handling**.
During the journey	
- Describes **water quality** aspects in detail including with reference to transport. - Makes the general recommendation to **monitor water quality** and adopt **mitigation measures**. - Identifies a few **water quality parameters** as a priority to monitor and maintain. - **Describes** the relationship between **key water quality parameters and the needs of fish**.	-
Post-transport	
- Makes recommendations on **monitoring and recording appetite, swimming behaviours and mortalities** for several days after unloading.	-

5.3. Third Party Certification Standards

Table 3: Criteria in two third party certification standards

GlobalGAP	Naturland
Pre-transport	
- Requires cleaning and **disinfecting**. - Requires **transporters to be identified** to allow traceability. (all stages)	- Requires **recording** of timing of all processes, equipment and substances used. (all stages) - Makes the general requirement for those carrying out procedures to have **expertise**.
Loading and unloading	
- Encourages **gentle handling**.	- Makes the general requirement that **equipment** must be free of any risk of causing injury.
During the journey	
- Makes the general requirement to **control oxygenation**. - Makes the general requirement to have routine **water quality monitoring and control** programme based on a risk assessment.	- Gives maximum **stocking density**. - Gives maximum road and wellboat **journey durations**, and maximum duration without water exchange. - Makes the general requirement that enough **oxygen** be supplied during transport. - Makes the general requirement that **procedures must be done quickly**. (all stages)
Post-transport	
-	-

6. RECOMMENDATIONS

These recommendations are based on the empirical evidence of welfare concerns during the live transport of fish that are detailed in this report, taking into account the international standards established by the OIE and the knowledge that has been developed since the OIE standards were developed. They also take account of standards and guidelines developed by Member States and by independent and sector initiatives, and of legal requirements in Norway.

It is recommended that EU legislation be updated to reflect current knowledge on fish needs and on transport methods, in order to minimise the suffering of fish and to facilitate a level playing field in EU production that exceeds OIE standards.

6.1. Pre-transport

6.1.1. Vehicles, vessels and operators

A licencing regime should be created to ensure that vehicles are designed, constructed, equipped and maintained to safeguard the welfare, health, and safety of the animals. Key aspects of this include monitoring and maintaining water quality and biosecurity at every stage, and avoiding injury to the fish.

Operators should have control systems for: maintaining the competence of personnel, cleaning and disinfecting regimes, maintaining vehicle and equipment, exchanging water, handling dead fish, and record keeping. Training should include the species' physiology, its needs and behaviour, and understanding of stress and disease, and how the species react to stress or infection

Vehicles and vessels should have the equipment to carry and supply supplemental oxygen, to continually monitor oxygen, CO^2, temperature and (if relevant) salinity with real time monitoring from the cab, and carry equipment for monitoring pH and total ammonia levels. Ventilation must be sufficient for removing carbon dioxide, and the vehicle should have a known water volume capacity.

Equipment and systems should be suitable to the vehicle and the journey. Long distance containers should employ chillers, CO^2 degassing equipment, surface skimmers, water buffers, circulation pumps, and oxygen sources. Short-haul tank trucks should use chillers or ice (animals should never contact ice directly), circulation pumps, anti-foam agents, and oxygen sources.

Containment units, fittings and equipment should have smooth surfaces and no holes, cracks or sharp angles, which may injure the fish or make effective cleaning and disinfection difficult. Exit valves should be large enough to allow the fish to exit without being physically injured. Pumps and pipes installed with closed systems should be sealed and not suck in air. No equipment should emit harmful substances.

Vehicles should have devices or materials to dampen vibrations and limit their transfer to transportation units, and have grills, caps and hinges as necessary to make inspection and cleaning and disinfection of every area effective. Wellboats and towing vessels should be equipped with a satellite tracking and monitoring system.

6.1.2. Journey preparations

Preparing fish for the journey typically includes a period of starvation prior to loading and this period should be as short as possible and necessary to clear the gut so as to maintain water quality. Species-specific maximum starvation periods should be established, taking water temperature into account.

In some cases a sedative can be used under the guidance of a veterinarian to calm fish for the journey. Reducing the water temperature is beneficial for salmonids and must be done at a maximum rate of change of 1.5°C/hr and to a minimum temperature of 6°C. Rainbow trout benefit from dilute salt solutions or a current during, before or after transportation. Feeding rainbow trout glucan in low doses or tryptophan-supplemented feed for several days or weeks prior to transporting can reduce related effects of stress.

Fish can be pre-conditioned to cope with crowding and harvesting by repeated stressing before netting. These procedures must be especially gentle to avoid inducing more stress.

Fish should be inspected for fitness to transport and should not be loaded if showing signs of disease, physical damage, or unusual behaviour, or if they have recently been exposed to a significant stressor.

Operators should plan for the presence of sufficient staff, the maintenance of water quality parameters including carrying 50% more oxygen than required for the intended journey, assess risk factors along the journey (including weather conditions for vessels), and have contingency plans in place to prevent and, if necessary, tackle acute events. Water quality, temperature, journey length, species, life-stage, and transport system must especially have been taken into account in planning the density of fish to be loaded. Use of open systems should be planned especially with biosecurity in mind. A person should be identified as responsible for recording information.

Water quality sensors should be checked and calibrated. In closed systems, supersaturation with gaseous nitrogen must be checked and water degassed before loading if necessary.

6.2. Loading

The overall loading time should be kept to a minimum and the best handling practices developed by the EU Platform on Animal Welfare Voluntary Initiative on Fish Welfare (EUPAW, 2020) should be implemented. The principles of good handling apply equally during loading and unloading.

Crowding should be carried out in steps and without invoking a significant stress response.

The use of fish pumps is preferred to the use of nets for moving and loading fish. Use of pumps requires monitoring of the water speed and pressure, that fish are exiting pipes gently, and for any injuries. Use of nets requires not-overloading the nets, minimising time out of water, and monitoring for injuries. Nets should be knotless, and the sides of braille nets should be lined to reduce injuries. Automated fish counters, and when available biomass estimating equipment, should be applied.

Used farm water should be separated from the fish. They should be loaded into water that is controlled to have low levels of metabolites, metals and organic matter. Supplementary oxygen should be added to the water throughout the loading period.

Any containers should be checked that they are secure enough to prevent their displacement during the journey.

6.3. Transportation

The fish should have settled in their transport units and be exhibiting calm behaviour before starting the journey.

During the journey gentle driving is necessary to minimise a range of stress related impacts. No feed should be offered during the journey except during exceptional journeys of over two days.

Water quality parameters should be kept within acceptable limits throughout the journey, and supplemental oxygen should be supplied especially during the first hour of transport. Changes in water temperature should be avoided.

Preferably, fish should not be inspected manually during the journey. Installation of underwater cameras should be promoted. Especially on shorter journeys, containers should normally only be opened when monitoring equipment indicates that water quality parameters cannot be maintained. Fish must be accessible for inspection at all times.

Fish can be transported in plastic bags with water and pure oxygen at a ratio between ½ and ⅓ for a maximum of 12 hours. Bags should be extra strong or double bagged and have rounded or tied off corners. Bags should lie flat and at right angles to the direction of travel (except high-backed ornamental species which should be placed upright). In this case the vehicle does not need the fixtures and fittings of other transport methods. Stationary periods should be avoided during the journey, and should be a maximum of 20 minutes for salmonids and 60 minutes for cyprinids. Acclimation is achieved by placing plastic bags into the receiving water bodies for a sufficient period before opening them. The use of salt water (3g/L) in plastics bags is recommended with carp. Research is recommended to adapt for the European context the addition of banana stems to the transport bags of catfish fingerlings to reduce mortality by activating the immune system.

The operator should inform the recipient of any delay or change during the journey.

Key water quality parameters should have limits established that are specific to the species and life-stage of the fish.

Table 4: Water quality parameters that are generally applicable across species

	Minimum	**Maximum**	**Observation**
Oxygen (saturation)	70%	200%	Max 100% if transported by air
Carbon dioxide	-	5-10mg/L	
Ammonia (NH^3)	-	0.1mg/L	

Source: summarised from Wedemeyer, G.A.,1996, Physiology of Fish in Intensive Culture Systems, Springer

6.4. Unloading

The overall unloading time should be kept to a minimum and the best handling practices developed by the EU Platform on Animal Welfare Voluntary Initiative on Fish Welfare (EUPAW, 2020) should be applied. The principles of good handling apply equally during loading and unloading.

Prior to unloading, the fish should be acclimated to the receiving water and should be exhibiting calm behaviours.

Fish should be monitored for signs of injuries during and after unloading. Some species, including trout, benefit from storage in an outlet aquarium for several days after transportation.

The stress of transfer from fresh- to seawater can be ameliorated by a sedative under the guidance of a veterinarian.

6.5. Post-transport

The transport is likely to affect the fish for days after unloading. Feeding should be resumed only when the animals demonstrate appetite. Monitoring of appetite, abnormal behaviours, the emergence of disease, and increased mortality, should continue for one week after unloading. In the case of increased mortality during the days after transport a health check should be carried out by a veterinarian or fish health expert. It is recommended to let trout and carp recover in salt water (3g/L).

Reporting of journey records should allow monitoring and continual improvement of the implementation of welfare standards, the vehicle licensing regime, and operator competences. Information should be recorded by the transport operator, reported to the competent authority, and made available to the dispatcher and recipient. Essential information includes: journey start and end time; the species, size, and number of animals loaded; the number of animals injured and number of mortalities from the start of loading to the end of unloading, and the cause of injury or mortality; the route including other animal holding sites visited; time and place of any release or intake of water; consumption of oxygen; key water quality parameters and especially temperature before loading, after loading, at regular intervals during the journey, and in containers and the receiving water before unloading; the time of disinfection and the disinfectants and methods used.

6.6. Special Note on Turbot

Research is required to further develop live transport techniques for turbot. Cold acclimation at 2°C for 2 days followed by waterless transportation with high oxygen and at low temperatures is necessary to reduce respiration, metabolism and problems associated with transport of live turbot. These conditions change several physiological systems including immune and liver function and may result in death from the inability to excrete metabolites when out of water.

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ANNEX – TRANSPORT WITHIN EU AQUACULTURE PRODUCTION SYSTEMS

The effects of the different stressors related to live fish transportation greatly depend on the transport system and equipment as well as on the capacity to cope with stressful conditions and biological requirements of each species, as well as of the different developmental stages (Table 1). Therefore, the most relevant aspects of aquaculture production and transport systems related to determining the timing and nature of live transports of the seven most produced species in European aquaculture are detailed below:

Table 1: Transport methods used for the transfer of Atlantic salmon, rainbow trout, gilthead seabream, European seabass, common carp, African catfish and turbot at different stages of the rearing cycle.

Transport 1 (T_1) *= larvae fish transferred from hatchery to nursery systems;* ***Transport 2 (T_2)*** *= alevine fish transferred from nursery to on-growing systems;* ***Transport 3 (T_3)*** *= juvenile/adult fish transferred from on-growing systems to slaughtering or to market (alive);* ***Transport 4 (T_4)*** *= selected adults transfer to breeding systems.* ***Transport 5 (T_5)*** *= wild specimens transported to farming systems. NA = not applicable***.**

	Transport 1	**Transport 2**	**Transport 3**	**Transport 4**	**Transport 5**
Atlantic salmon (*S. salar*)	Road	Road Well-boat Helicopter	Road Well-boat	Road	NA
Rainbow trout (*O. mykiss*)	Road	Road	Road	Road	NA
Gilthead seabream (*S. aurata*)	Road	Road boat	NA	Road	Boat Road
European seabass (*D. labrax*)	Road	Road boat	NA	Road	Boat Road
Common carp (*C. carpio*)	Road	Road	Road	Road	NA
African catfish (*C. gariepinus*)	Road	Road Aeroplane	Road	Road	Direct
Turbot (*S. maximus*)	Road	Road Well-boat Aeroplane	NA	Road	Road

Atlantic Salmon (*Salmo Salar*) and Rainbow Trout (*Oncorhynchus Mykiss*)

Atlantic salmon and rainbow trout farming is generally intensive and mainly destined for consumption purpose. The intensive system is characterised by a high production at high fish densities where many parameters are under human control. The most common farming systems used are flow-through systems, Recirculating Aquaculture Systems (RAS), net cages, and semi-closed containment systems (S-CCS). At intensive breeding systems, selected broodstocks are held in large fresh water ponds or tanks (usually flow-through or RAS systems) where they release eggs and milt, which will be mixed to produce fertilized eggs. The fertilised eggs are then placed in purpose-built incubators until hatching. After hatching, fry absorb nutrients from a yolk sac attached to their bodies, and they remain in the hatching environment until they are able to feed independently. Then, larvae fish are directly transferred to first-feeding tanks (T1) using nets and buckets, pumps or transported by road to nearby facilities.

At the nursery stage, Atlantic salmon and rainbow trout have different rearing requirements that will dictate the type of containment, but the source of water available will determine whether flowthrough, semi-closed containment systems or recirculation systems are best. Normally, Atlantic salmon are kept on land in freshwater tanks after hatching, before smoltification starts naturally or is induced artificially. The smolts or post-smolts are then transferred (T2) mostly to sea-cages, or RAS and S-CSS systems by road truck and/or well-boats, or eventually by helicopter, for the final grow-out phase until harvest. In rainbow trout, fry are moved to outdoor grow-out facilities (T2) using road trucks and/or well-boats, which can comprise concrete raceways, ponds, cages in lakes or sea-cages with different sizes and characteristics according to site availabilities, environmental conditions, and specific company targets. Nowadays, RAS systems are becoming popular and both species can be also transferred from nursery systems by road trucks (T2) for on-growing in this systems until being harvest. Atlantic salmon and rainbow trout are grown on to a marketable size usually within 9 months in sites dedicated to producing portion-size trout of 450 g average weight. Some fish, though, are grown on to larger sizes over 20 months and would be harvested at 3 kg plus. In addition, small-scale rainbow trout farms can use semi-intensive systems for on-growing where young stock is brought in by road (T2) and grown-out for either food or re-stocking markets (T3). Extensive salmonids production is quite rare at commercial scale, and mostly consists of releasing juvenile fish for downstream migration at the smolt stage.

As mentioned before, prior to any transportation or harvest, salmon and trout are fasted and crowded to enable them to be pumped or brailed either into transport containers or to a mobile stunning and slaughter facility. Following transport for harvesting, by road trucks and/or well-boats (T3), the fish are again pumped over distances that may range from a few metres to more than a kilometre. The fish may be held in lairage following transport, but before stunning and slaughter, both to rest the fish and to optimise the use of the slaughter facility. In some systems, the fish are cooled either during well-boat transport or during a holding period prior to stunning (Lines and Spencer, 2012). The transport of salmonids (smolts and adults) among facilities and for slaughtering, as well as during changes of net and some sea lice treatments (in case of farming at sea), is likely to have serious welfare implications (Santurntun et al. 2018).

Gilthead Seabream (*Sparus Aurata*) and European Seabass (*DicentrarchusLabrax*)

Gilthead seabream and European seabass can each be produced in three different aquaculture systems, whose techniques and procedures are very similar for the two species. Intensive systems are characterised by a high production at high fish densities, where many parameters are under human control. To secure a reliable and sufficient supply of good quality fish eggs, most hatcheries have established their own broodstock units, where breeders of different age groups are maintained under long-term stocking conditions. Parent animals may come from the wild (T5), but nowadays most of them come from a selective programme at the farm (T4). After hatching, the larvae will absorb their yolk sac and, once they start feeding, weaning usually takes place in a dedicated section of the hatchery (T1) (i.e. nursery area) equipped with larger round or rectangular tanks (10-25 m^3 approximately). Juveniles are pre-fattened intensively with a controlled diet and at high densities until they reach the size for the on-growing phase. In intensive production, on-growing units are supplied with juveniles which may be purchased from separated hatcheries (T2), but large production units normally rear their own. Intensive on-growing phases can be carried out in land-based installations or in sea- cages (T2). Semi-intensive farming systems are usually carried out in net enclosures within limited areas of the lagoons or in earthen ponds, where human control of the farming environment is much lower than in intensive systems but greater than in the extensive ones. This technique involves artificial enrichment with fry collected by specialised fishermen (T5) or seeding with pre-fattened juveniles in intensive systems to minimise mortality and shorten farming time (T2). Extensive systems are based on the natural migration of euryhaline fish between the open sea and coastal lagoons, brackish ponds or salt marshes, and they have been widely developed in northern Italy ('vallicoltura') and in southern Spain ('esteros'). This traditional extensive method of lagoon management places special traps or barriers made of reeds, nets or cement in appropriate lagoon sites to capture fish during their autumn migration to the open sea. During the production cycle, no transport is required.

Before transport and regardless of the system, both species are fasted and then crowded before collection by brail or by pumping (Lines and Spencer, 2012). Seabass and seabream are seldom transported more than a few tens of metres (if at all) from the on-growing facility to the stunning or killing point (European Food Safety Authority (EFSA), 2009).

Common Carp (*Cyprinus Carpio*)

Cyprinus carpio is a freshwater species that is generally reared in ponds in intensive, semi-intensive, or extensive monoculture or polyculture systems, or in integrated carp culture with other agriculture systems (Peteri, 2004; EFSA, 2008). Spawning can either occur in 1) large ponds, where fry can be harvested or left there until they reach fingerling size and are moved to prepared ponds (T1), 2) hatcheries, where ovulation and spermiation are artificially induced and eggs are artificially fertilised, then fry are moved from tanks into ponds (T1) when they reach the feeding fry stage (Peteri, 2004). Fry are nursed in ponds or alternatively, if predators are present near the ponds, in tanks or in industrial raceways or water recirculating systems (Peteri, 2004). Then fingerlings production takes place in semi-intensive ponds, and from there they can be moved to ongrowing systems (T2). The ongrowing phase where carps reach market size can take place in 1) extensive monocultural production systems in stagnant water ponds; 2) in intensive monocultural production systems in cages, irrigation reservoirs, running water ponds/tanks, or in recirculating systems; 3) in polycultural systems with other species;

or 4) be integrated with animal husbandry and/or plant production (Peteri, 2004). From here they can either be transported (T3) to be sold live to consumers or restaurants, or to be slaughtered in an abattoir (IBF et al., 2017). Broodstock can be transferred between ponds and the hatchery (T4) either in a vehicle with an oxygenated fiberglass container or manually using a double or single-space hammock designed to transport fish (Horváth et al., 2015).

In common carp, transportation events will depend on the type of facilities. Some facilities can hold all the production stages, and thus, transportation between production stages will be minimal and will only take place within the facilities. Other facilities will only cover one or two production stages and transportation to other facilities will be needed to complete the production chain (IBF et. al, 2017). During harvesting, before transportation, fish are pre-conditioned by repeated stressing before netting (Peteri, 2004). Any sick or injured fish is not transported, and fish are feed deprived for up to 7 days (in winter) to prevent deterioration of water quality during transportation (IBF et al., 2017). Water level is decreased in ponds and fish are collected by crowding and brailing (Lines and Spence, 2012), and caught manually with nets, being out of the water for 5 s to 2 min (IBF et al., 2017). They are manually loaded into closed-system aerated tanks in insulated trucks either directly from the nets or from baskets or metal containers, and can be out of water from 5 s to 5 min (IBF et al., 2017). The duration of the transportation event ranges from 0.5 to 12 h, and temperature, pH, and oxygen levels are generally monitored (IBF et al.., 2017).

African Catfish (*Clarias Gariepinus*)

Clarias gariepinus is reared in ponds, small pits or ditches, concrete raceways, fiberglass or plastic tanks, or cages, in various levels of intensification (Pouomogne, 2010). Other systems used in this species are Intensive Recirculating Aquaculture Systems (RAS) and Nile tilapia polyculture ponds (Pouomogne, 2010). The production cycle starts with broodstock receiving hormonal treatments to induce ovulation and spermiation. Then, the eggs are collected and fertilized and the larvae are kept in tanks. Alternatively, in semi-natural enclosures, wild fish naturally populate ponds after flooding. After vitelline resorption the larvae are transferred into nursery ponds (T1), where fingerlings are graded before being transferred to production ponds (T2). One method of transporting fingerlings and fry are plastic bags with water and oxygen (Nasrullah et al., 2021), and sometimes these transportation events take place by airlift between countries, with flight times of up to 16h (Basharat et al., 2020). Once they reach market size, ponds are harvested using hauling seines and the fish are transported (T3) to be sold directly to consumers or to retailers (Pouomogne, 2010) or are sent to a killing facility in dry containers where they are chilled or electrically stunned and killed by decapitation (Lines and Spence, 2012).

Turbot (*Scophthalmus Maximus*)

Turbot aquaculture is more recent in terms of history compared to the species above. S. maximus farming started in the 1970s in the United Kingdom and subsequently established in France, Spain, and Portugal, expanding nowadays through other European and world-wide countries. However, the production level of farmed turbot is relatively low compared to other fish species. Turbot is mostly reared in intensive conditions (Rodriguez-Villanueva and Fernández Souto 2005). Broodstock are usually maintained in concrete or cement tanks, with volumes ranging from 20-40 m^3 at densities of 3-6 kg/m^3, where they are stripped for spawning. The fertilized eggs collected and kept in incubators (small aerated tanks) for hatching. Then, hatched larvae are distributed (usually by small-scale road transports) to hatchery-rearing systems (T1). Once they reach a certain size, they are transferred by road to nursery systems (T1). Turbot are nursed in square or circular tanks (10-30 m^3) with open-circuit pumped seawater. After this period (duration 4-6 months), turbot can reach from 10 to 100 g, being transported then to world-wide on-growing systems (T2) by road trucks and/or well-boats, depending whether they are either reared in on-shore tanks (the most common technique for this species) or flat-bottomed cages. In such closed transportation, water is supplied with oxygen and is ventilated, and the fish is cooled to approximately 10 °C. Long-distance road/sea transportations of juveniles (1-3 g) can be substituted by air transport (e.g. from Spain to Norway) where weight limitations apply. During air transportation, turbot is transported in a moisture environment without water, however with temperature control (Norwegian Scientific Committee for Food Safety 2008). Onshore tanks are usually shallow and square or circular cement tanks (25-100 m^3) with open-circuit pumped seawater and aeration or oxygenation systems (Lines and Spencer 2012). Eventually, flat-bottomed sea-cages (with a metal or netting bottom) can be used. Turbot can be also reared in semi/extensive conditions in ponds, which is characterized by low stocking densities, where road transport occurs. Conversely, turbot are recently reared in superintensive recirculating (RAS) systems (Li et al. 2013). Turbot takes about two years to grow to a market size of approximately 1 kg from fertilized egg. Once they reach a marketable size, turbot is deprived of food for some days, crowded using nets or partitions in the tanks and then collected by hand brail, removed from the water and placed onto ice (slaughtering by thermal shock) for transport by road to a slaughtering and processing plant. Although breeding techniques and genetic selection have improved during last years (T4), broodstock and juvenile supply from the wild (T5) is still punctually needed to improve the fry quality and increase genetic diversity in the stock. Wild turbot are usually captured by gill-nets and transported (by sea and road) in small containers or plastic bags with fresh sea water to onshore facilities (Pyanov 2021).

The purpose of this study is to analyse the particular welfare needs in live animal transport of aquatic animals. The in-depth analysis describes the key causes of suffering in relation to the needs of farmed fish, and explores strengths and weaknesses in the EU regulation and in current guidelines. Recommendations are made to mitigate the many welfare challenges identified in the study.

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