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TECHNICAL REPORT

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# (Very) long-term transport of *Silurus glanis*, *Charcharhinus melanopterus*, *Scomber colias*, *Trachurus picturatus*, *Polyprion americanus*, *Rhinoptera marmoratus*, *Salmo salar*, *Scomber scombrus*, *Sardina pilchardus*, and others, by land, water and air

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## Abstract

In this paper, we cover 4 years of live fish transports that ranged from 14 to 200 h (8 days), and bioloads from 3.8 to 76.9 kg/m$^3$. The key ingredients for success in all trips, where virtually no mortality occurred, was atributed to (1) pre-buffering the water with sodium bicarbonate and sodium carbonate at 50 g/m$^3$ (each)—and/or ATM Alka-Haul™ at 25 g/m$^3$—and applying additional (partial or full) doses throughout each transport, whenever the tanks were accessible; (2) pre-quenching ammonia with ATM Triage™ at 32 g/m$^3$, and applying additional (partial or full) doses throughout each transport, whenever the tanks were accessible; (3) keeping the dissolved oxygen saturation rate above 100%, ideally above 150%; (4) Keeping temperature on the lower limit of each species' tolerance range; (5) Using foam fractionators to effectively eliminate organic matter from the water and (6) Using pure sine wave inverters, which allows for a steady supply of electrical current throughout the transport. The use of a 'preventive' versus 'corrective' pH buffering philosophy is also discussed.

KEYWORDS

pH buffering, ammonia quenching, foam fractionator, protein skimmer, voltage inverter

## 1 | INTRODUCTION

Historically the transport of live fish has proven difficult, since water quality inexorably deteriorates with time if no measures are taken to prevent it. The higher the bioload of animals moved or, conversely, the lower the volume of water, the more rapidly physical and chemical parameters, such as oxygen, pH, and ammonia, will deteriorate. At the end of the XX$^{th}$ century, most transports still relied on crude filtration systems, with practically no monitorization of water quality parameters, which has been described in often colourful autobiographic works, such as Correia (2015, 2016, 2017, 2019) and Powell (2001). In recent years, however, long-term transport methods for aquatic organisms have seen rapid improvements, which has even allowed for the maintenance under human care of species once considered 'impossible', such as *Carcharodon carcharias* (Ezcura et al., 2012), *Hydrolagus colliei* (Correia, 2001), *Sphyrna lewini* (Young et al., 2002), *Mobula mobular* (Correia et al., 2008), *Mola mola* (op. cit., Hays et al., 2020; Howard et al., 2020), *Sarda sarda* and *Scomber scombrus* (Correia et al., 2011), *Coryphaena hippurus* and *Coryphaena equiselis* (Rodrigues et al., 2013), *Prionace glauca* (Baylina et al., 2017) or even *Naucrates ductor* (Correia et al., 2018). Other relevant works in this

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field include classic compilations of multiple transport methods, such as Smith et al. (2004) and Correia and Rodrigues (2017).

This paper reports on advancements made in the aforementioned methods, allowing for full survivorship in transports by land, air and water, that lasted as long as 8 days.

## 2 | COLLECTION

A large *Silurus glanis* was collected from the Ebro river, in Tauste (Spain), and was held at the Acuario de Zaragoza (Spain) for approximately 1 year before shipping to Wonders of Wildlife in Springfield. During that time it was kept in a 10,000 L round holding tank, which had a 25% water change done daily. When collected, the animal was 232 cm total length (TL) and 125 kg.

Two *Carcharhinus melanopterus* were born at Sea Life Center Oberhausen, in Germany, on 2 July 2015, where they were kept until 8 September 2016, when they were moved by road to Oceanário de Lisboa, in Lisbon. One animal was 95 cm TL, weighing 4.5 kg, while the other was 96 cm and weighed 4.8 kg.

The *Rhinoptera marmoratus* were wild-caught in southern Turkey and kept in Istanbul Akvarium at 21.5°C until being moved to Atlantis Aquarium Xanadu in Madrid, by road and water.

The *Salmo salar* were moved from aquaculture facilities in Sveio (Norway), Letterfrack (Ireland) and Galway (Ireland), in 2018, 2019 and 2021, respectively. The Norwegian and Galway fish were kept in open systems with running natural freshwater that, on departure, was at 4.7°C (Norway) and 10.0°C (Galway). The animals from Letterfrack were kept in a floating cage at Lough Fee and were loaded at 7.4°C.

The *Sardina pilchardus* were collected off La Ciotat, in the south of France, near Marseille, with a purse-seiner, with the animals being removed from the ocean using small smooth hand-held landing nets. They were then kept on shore in a 2 m wide tank running on a flow-through system with natural seawater.

The *Polyprion americanus* were also collected off La Ciotat, while diving with hand-held landing nets. Once on shore, they were kept in a 5 m wide tank running on the same flow-through system with natural seawater used for *S. pilchardus*.

The *Scomber colias* and 2018 batch of 1700 *Trachurus picturatus* were collected using a similar method as for *S. pilchardus*, but off the island of Faial, in the Azores archipelago, with removal from the ocean done with hand-held landing nets, lined with a vinyl cover, to minimize abrasion. The 2021 batch of 252 *T. picturatus* were caught by hook and line. All specimens were kept in a 2 m wide tank with 4 $m^3$ water volume running on a semi-open system consisting of UV and sand filter, with a turnover of 1 times/h.

The *Seriola dumerili*, *Seriola rivoliana* and *Trachinotus ovatus*, were collected by hook & line and were kept in an 8 × 4 m elliptical tank with 40 $m^3$ water volume running on a semi-open system consisting of a sand filter and UV, with a turnover of 0.3 times/h and a foam fractionator with a turnover of 0.3 times/h.

The *Balistes capriscus*, *Boops boops*, *Mycteroperca fusca* and some *Diplodus vulgaris* were also collected off the Faial coast by specialized divers with hand nets. These animals were kept in 2 m wide tanks with 4 $m^3$ water volume, equally running on a semi-open system equipped with a sand filter and UV sterilizer.

The *S. scombrus*, *Myliobatis aquila*, *Dasyatis violacea* and *Pteromylaeus bovinus*, were collected in a set-net operating off Olhão, in the southern Portuguese shore. This commercial fishing apparatus targets *Thunnus thynnus*, but traps multiple additional species as well, including other members of family Scombridae, such as *S. scombrus*, *S. sarda*, *Euthynnus alleteratus*, *Auxis rochei* and *Katsuwonus pelamis*. This is a passive fishing method, where fish's trajectories are blocked by a barrier net that runs north to south, perpendicular to the shoreline, for 3000 m. Whether the animals are swimming eastwards, into the Mediterranean, or westwards, into the open Atlantic, the barrier net drives them into a large open bag known as 'ascension', which funnels into a second bag, know as 'playground'. It is inside this bag—which is 100 m long × 60 m wide and 30 m deep, that the fish swim freely until this compartment is hauled, twice daily. Once the compartment is reduced to a cube that's approximately 5 m wide, long and deep, a second—smaller—net is placed along the bottom, trapping all fish inside as it is hauled to the surface, with the help of a crane. At this point the collection method becomes identical to that described for *S. pilchardus*, as a vinyl bag collects approximately 100–200 *S. scombrus* —in 200–300 L of water—and is pulled from the ocean (Figure 1). The bag is then slowly immersed inside a 2.0 m wide transport tank, placed on the deck of the fishing vessel. This tank is equipped with an oxygen cylinder, which keeps the oxygen saturation rate above 100%, while new seawater is pumped continuously in, overflowing the tank (Figure 2). The elasmobranch species (i.e., *M. aquila*, *D. violacea* and *P. bovinus*), were typically collected from this smaller area of the set-net as well, using hand-held scoop nets.

Once on shore, all animals collected in Olhão were moved from the collecting tank to the holding tanks using plastic bags (Figure 3). The holding tank ran on a flow-through system with natural seawater added continuously, equipped with sand filter, biotower, and a rack of 12 ultraviolet (UV) lamps.

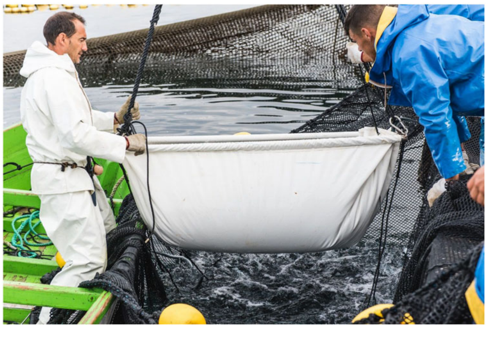

**FIGURE 1** Collecting *Scomber scombrus* from a commercial set-net off Olhão, in the south of Portugal, with the assistance of a vinyl bag holding approximately 200 L of water and 200+ fish. Photo by Janos Debreczi [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 2** Collecting *Scomber scombrus* from a commercial set-net off Olhão, in the south of Portugal, with the assistance of a vinyl bag holding approximately 200 L of water and 200+ fish. The fish are placed inside a 2 m wide fibreglass tank, where pure oxygen and seawater are added continuously. Photo by Janos Debreczi

**FIGURE 3** Collecting *Scomber scombrus* from a commercial set-net off Olhão, in the south of Portugal. The fish are moved to the holding tank, on shore, using plastic bags. Photo by Janos Debreczi [Color figure can be viewed at wileyonlinelibrary.com]

The *Helicolenus dactylopterus*, *Scorpaena notata*, *Diplodus cervinus* and some *D. vulgaris* were collected off Peniche, Portuguese western shore, by hook and line, and kept in a 4000 L closed system fed by a borehole, with filtration consisting of a Tropical Marine Center™ 5000 kit, which includes a foam fractionator, fluidized bed sand filter, 8 UV lamps and a biotower. The *Maja squinado* was purchased from a shellfish dealer and had originally been caught by bottom gill-nets.

The *Argyrosomus regius* were captive bred at IPMA, a research facility also in Olhão, south of Portugal.

## 3 | TRANSPORT

Details on transports are given in Table 1, which includes the number of specimens per tank (or bag), their individual weight and volume of water, which allowed for the calculation of bioload, that is, the biomass divided by cubic metres of water.

With the exception of the *Silurus* customized tank, and plastic bags used for *S. colias*, *P. americanus* and *T. picturatus*, all transports were done in round polyethylene tanks (diameters and volumes indicated in Table 1), with a fibreglass reinforced wooden lid on top, which was fitted with one Laguna™ Pressure-Flo™ 12000 Pond filter, inside which was a set of four foam disks, a mesh bag with 500 g of activated carbon and a UV lamp in the centre; each filter was powered by a 220 V Laguna™ Max-Flo™ 14000 pump, which moved 14,000 L/h. Each tank was also fitted with an AquaC™ foam fractionator, also powered by a 220 V Laguna™ 14000 pump. The 220 V AC current that powered all pumps was drawn from Xunzel™ pure sine wave inverters model 2000 (Figure 4), which were connected to either the 12 V DC battery of a van used for transporting the 1.75 or 1.90 m wide tanks, or the 24 V DC batteries from a truck, used to move the 2.4 m wide tanks. All tanks were fed pure oxygen from a medical grade compressed oxygen cylinder, normally at a rate of 1–2 L/min, and air was also supplied from a standard 220 V blower.

During all transports, sodium bicarbonate and sodium carbonate were added to the water upon packing, to buffer pH, at 50 g/m$^3$ (each). ATM Triage™ was added as well, at 32 g/m$^3$ to quench active ammonia. In some of the most recent transports, ATM Alka-Haul™ was also added, at 25 g/m$^3$. Subsequent additions were then made during water quality checks (with the exception of transports by air in plastic bags), typically at 4 h intervals, with the objective of keeping pH stable throughout each trip. Likewise, any ammonia readings higher than 0 mg/L were counteracted with 100% of the original dose of ATM Triage™, while measurements of 0 mg/L were traditionally met with alternated additions of 25% or even none of the original dose.

Oxygen and temperature were also recorded at each water quality check. Oxygen was measured using a hand-held OxyGuard™ Handy Polaris probe, while pH and temperature were measured with a hand-held Hanna™ pHep®5 probe, and ammonia was tested with a Fluval™ test-kit.

The *S. glanis* was moved in a custom built rectangular box, which was as long as an aircraft pallet would allow (300 cm long × 80 cm wide × 120 cm high), to maximize the volume of water carried, which was 1625 L. The tank (Figures 5 and 6) included a 30 cm high baffle well, to avoid sloshing of the water. It was also fitted with a small battery operated aeration unit, which pumped air continuously into the water. While on the ground, or during the road transport from Zaragoza Aquarium to the airport in Madrid, and also from Chicago airport to Wonders of Wildlife, in Springfield Missouri, oxygen was added to the water through a 4 mm thick airline. Mechanical filtration consisted of a mechanical Aqua-Systems™ pleated laminated paper cartridge filter (Figure 7), with 1 kg of activated carbon inside, which was powered by a 12 V bilge pump mounted in the corner of the tank, protected from the large fish by a diagonal drilled plate. The pump was powered by three 12 V deep-cycle batteries, mounted in parallel. While on the ground, a charger allowed for the batteries to be charged from a standard 220 V outlet, while running the pump.

The *S. colias* were moved from Horta (Faial Island, in the Azores Archipelago) to Tel-Aviv by air, via Lisbon and Frankfurt, and then by road to the Jerusalem Aquarium. The fish were

**TABLE 1** Technical details on road, water and air transports from 2016 until 2021

| Date | Species | Source | Ind. weight (kg) | Tank width (m) | Tank volume (L) | No. ind./tank | Bioload (kg/m$^3$) | Duration of transport (h) | Origin | Destination | Refrigerated | Means |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4/14/2016 | *Silurus glanis* | wc | 125.000 | 3.0 × 0.8 × 1.2 m (rectangular) | 1625 | 1 | 76.9 | 46 | Zaragoza, ES | Springfield, MO, US | No | Road and air |
| 9/8/2016 | *Carcharhinus melanopterus* | cb | 5.000 | 1.75 | 1100 | 1 | 4.5 | 24 | Oberhausen, DE | Lisboa, PT | Van | Road |
| 7/24/2017 | *Scomber colias* | wc | 0.025 | 40 × 60 cm plastic bags | 6.5 | 2 | 7.7 | 36 | Horta, PT | Jerusalem, IR | Ice-packs | Air |
| 5/7/2018 | *Trachurus picturatus* | wc | 0.060 | 2.4 | 3600 | 616 | 10.3 | 200 | Horta, PT | Stralsund, DE | No | Water and road |
| | *Trachinotus ovatus* | wc | 0.120 | 1.9 | 1600 | 62 | 11.3 | 200 | Horta, PT | Stralsund, DE | | |
| | *Balistes capriscus* | wc | 0.120 | | | 4 | | 200 | Horta, PT | Stralsund, DE | | |
| | *Diplodus vulgaris* | wc | 0.100 | | | 70 | | 200 | Horta, PT | Stralsund, DE | | |
| | *Boops boops* | wc | 0.060 | | | 43 | | 200 | Horta, PT | Stralsund, DE | | |
| | *Diplodus cervinus* | wc | 0.300 | | | 1 | | 64 | Peniche, PT | Stralsund, DE | | |
| | *Helicolenus dactylopterus* | wc | 0.150 | | | 2 | | 64 | Peniche, PT | Stralsund, DE | | |
| | *Mycteroperca fusca* | wc | 0.800 | 1.9 | 1600 | 1 | 6.4 | 200 | Horta, PT | Stralsund, DE | | |
| | *B. capriscus* | wc | 0.120 | | | 6 | | 200 | Horta, PT | Stralsund, DE | | |
| | *Myliobatis aquila* | wc | 0.500 | | | 1 | | 60 | Olhão, PT | Stralsund, DE | | |
| | *Pteromylaeus bovinus* | wc | 0.500 | | | 1 | | 60 | Olhão, PT | Stralsund, DE | | |
| | *Scorpaena notata* | wc | 0.200 | | | 6 | | 40 | Peniche, PT | Königswinter, DE | | |
| | *Dasyatis violacea* | wc | 3.000 | | | 2 | | 36 | Olhão, PT | Königswinter, DE | | |
| | *P. bovinus* | wc | 0.500 | | | 1 | | 36 | Olhão, PT | Königswinter, DE | | |
| 7/3/2018 | *B. capriscus* | wc | 0.120 | 1.9 | 1600 | 4 | 19.8 | 48 | Peniche, PT | Königswinter, DE | Van | Road |
| | *M. fusca* | wc | 0.800 | | | 1 | | 48 | Peniche, PT | Königswinter, DE | | |
| | *S. notata* | wc | 0.200 | | | 1 | | 48 | Peniche, PT | Königswinter, DE | | |
| | *Maja squinado* | wc | 1.000 | | | 2 | | 48 | Peniche, PT | Königswinter, DE | | |
| | *D. violacea* | wc | 3.000 | | | 2 | | 36 | Olhão, PT | Königswinter, DE | | |
| | *Polyprion americanus* | wc | 3.000 | | | 1 | | 28 | La Ciotat, FR | Berlin, DE | | |

**TABLE 1** (Continued)

| Date | Species | Source | Ind. weight (kg) | Tank width (m) | Tank volume (L) | No. ind./tank | Bioload (kg/m$^3$) | Duration of transport (h) | Origin | Destination | Refrigerated | Means |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *P. americanus* | wc | 0.800 | | | 6 | | 34 | La Ciotat, FR | Stralsund, DE | | |
| | *P. americanus* | wc | 0.800 | | | 5 | | 56 | La Ciotat, FR | Rotterdam, NL | | |
| | *P. americanus* | wc | 0.800 | | | 5 | | 74 | La Ciotat, FR | Boulogne-sur-mer, FR | | |
| | *P. americanus* | wc | 0.800 | | | 8 | | 96 | La Ciotat, FR | Peniche, PT | | |
| | | | | 80 × 120 plastig bag | 42 | 1 | 19.0 | 24 | Peniche, PT | Edinburgh, UK | Ice-packs | Air |
| 10/18/2018 | *Rhinoptera marmoratus* | cb | 5.000 | 2.4 | 3600 | 4 | 5.6 | 144 | Istanbul, TR | Madrid, ES | Truck | road |
| 2/28/2019 | *Salmo salar* | cb | 0.200 | 2.4 | 3600 | 300 | 16.7 | 72 | Sveio, NO | Aveiro, PT | Truck | Road and water |
| 11/15/2019 | *Scomber scombrus* | wc | 0.075 | 2.4 | 3600 | 910 | 19.0 | 14 | Olhão, PT | València, ES | No | Road |
| 12/5/2019 | *Sardina pilchardus* | wc | 0.025 | 1.9 | 1600 | 600 | 9.4 | 19 | La Ciotat, FR | Olhão, PT | Van | Road |
| 12/13/2019 | *Salmo salar* | cb | 0.100 | 2.4 | 3600 | 300 | 8.3 | 76 | Letterfrack, IE | Aveiro, PT | Truck | Road and water |
| 1/14/2020 | *S. scombrus* | wc | 0.110 | 1.2 | 500 | 100 | 22.0 | 26 | Peniche, PT | Montréal, CA | No | Road and air |
| 11/19/2020 | *Seriola dumerili* | wc | 1.600 | 2.4 | 3600 | 9 | 3.8 | 48 | Horta, PT | Ponta Delgada, PT | No | Water and road |
| | *Seriola rivoliana* | wc | 1.600 | 2.4 | 3600 | 9 | 3.8 | 108 | Horta, PT | Olhão, PT | | |
| 11/25/2020 | *Argyrosomus regius* | cb | 0.200 | 2.4 | 3600 | 200 | 11.1 | 15 | Olhão, PT | Sant Carles Ràpita, ES | No | Road |
| | | | 10.000 | 2.4 | 3600 | 17 | 47.2 | | | | | |
| 1/19/2021 | *Polyprion americanus* | wc | 5.000 | 1.9 | 1600 | 5 | 15.6 | 14+34 | La Ciotat+Le Croisic, FR | Galway, IE | Chiller unit | Road and water |
| 1/23/2021 | *Salmo salar* | cb | 0.060 | 1.9 | 1600 | 600 | 22.5 | 46 | Galway, IE | Vila Real, PT | Chiller unit | Road and water |
| 1/29/2021 | *T. picturatus* | wc | 0.030 | 55 × 90 cm plastic bags | 21.5 | 6 | 8.4 | 36 | Horta, PT | Branson, MO, USA | No | Air and road |

Abbreviations: cb, captive bred; wc, wild caught.

**FIGURE 4** A pure sine wave inverter converted 24 V DC (or 12 V DC) from a truck (or van) to 220 V AC, which powered the filtration units on all the tanks [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 5** Custom-built tank for moving a 125 kg *Silurus glanis* from Acuario de Zaragoza (Spain) to Wonders of Wildlife (Springfield, MO, USA) via a Madrid to Chicago direct flight [Color figure can be viewed at wileyonlinelibrary.com]

packed two per bag, which measured 40 cm wide × 60 cm high, each holding 6.5 L of water and an equivalent volume of pure oxygen. Sodium bicarbonate and sodium carbonate were added to the water, during packing, at double the usual dose, that is, 100 g/m$^3$ each and ATM Triage$^{TM}$ at 64 g/m$^3$. Four frozen bottles of water (33 ml each) were also added, to keep temperature low throughout the transport.

The *S. scombrus* moved by air to Montréal, were driven from Peniche to Lisbon airport inside a sealed 500 L tank (Figure 8) with no filtration, to which the regular doses of sodium bicarbonate, sodium carbonate and ATM Triage$^{TM}$ were added upon packing, that is, 50, 50 and 32 g/m$^3$, respectively. Oxygen was also fed to the tank while on the road and on the ground, at the airport. Once the tank was moved into the airport's 'air' side, oxygen was no longer fed, and a small 6 V battery operated aeration unit, placed inside the lid, was turned on and assisted in dissolving the pure oxygen above the water level through an

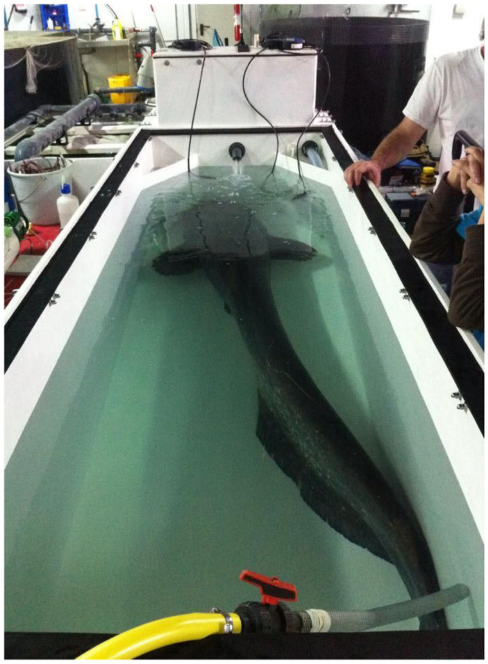

**FIGURE 6** Custom-built tank for moving a 125 kg *Silurus glanis* from Acuario de Zaragoza (Spain) to Wonders of Wildlife (Springfield, MO, USA). Note the effluent from the filtation unit returning to the tank above the *Silurus*' head. Once the lid was sealed, the water level was raised to approximately 10 cm from the top of the baffle well [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 7** Custom-built tank for moving a 125 kg *Silurus glanis* from Acuario de Zaragoza (Spain) to Wonders of Wildlife (Springfield, MO, USA). A cartridge filter consisting of laminated pleated paper, with 1 kg of activated carbon in the centre, was powered by a 12 V bilge pump [Color figure can be viewed at wileyonlinelibrary.com]

airstone placed at the bottom of the tank. The last measurement of dissolved oxygen, before the tank was sealed, showed 368.3% saturation.

Table 1 also provides details on cooling, marked as 'no' when it wasn't done, and otherwise marked as stated below:

**FIGURE 8** 500 L, 120 cm wide and 120 cm high tank, used to move 100 *Scomber scombrus* from Peniche (Portugal) to Montréal (Canada), via a Lisbon to Toronto direct flight

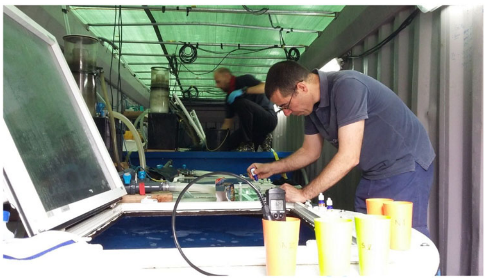

**FIGURE 9** Temperature, pH, oxygen saturation rate and ammonia concentration were tested approximately every 4 h on all transports by road or water. Effluent from protein skimmers was also dumped whenever required, during water quality checks [Color figure can be viewed at wileyonlinelibrary.com]

- 'Truck' or 'Van' means the tanks were moved in a refrigerated truck or van, where temperature was kept at the designated temperature.
- 'Chiller unit' means the filtration system was fitted with an Hailea$^{TM}$ chiller unit.
- 'Ice-packs' means that four frozen bottles of water (0.33 L) were placed inside the box.

Approximately every 4 h, which coincides with the time one truck driver needs to be relieved by his driving buddy, a water quality check was performed on all tanks moved by road (Figure 9). Each check included the collection of a 200 ml sample of water from each tank, from which 5 ml were then moved into a test tube, where ammonia testing reagents were added. Temperature, pH and dissolved oxygen were measured sequentially on all samples collected and results recorded on a sheet.

## 4 | RESULTS

Table 1 highlights the bioload and duration of each transport, which was counted from the moment the first animal was placed inside the tank (or bag), until the last one was removed. Mortality was virtually nil in all transports, and 2 *R. marmoratus* were in fact born immediately after arrival to Madrid.

While water quality parameters were mostly kept at their initial values using the protocol highlighted earlier, a selection of more relevant results is presented—with comments—in this section.

Temperature in the 24 h road transport of two captive bred *C. melanopterus* from Sea Life Oberhausen to Oceanário de Lisboa remained stable between 24°C and 25°C (Figure 10), although a rise at 18 h prompted the attendants to lower the temperature in the cargo cabin, which in turn provoked a drop to 23°C. This was counteracted by adjusting the temperature in the cargo section again to 24°C, which was the desired temperature on arrival.

Oxygen was kept at a mean 160% saturation throughout the transport (Figure 11), which was the standard in all road and water transports highlighted in Table 1.

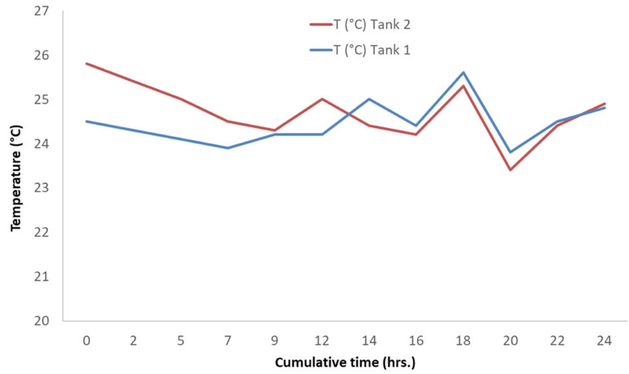


**FIGURE 10** Temperature in two tanks where two captive bred *Carcharhinus melanopterus* (95 and 96 cm) were moved from Oberhausen (Germany) to Lisbon (Portugal) over 24 h

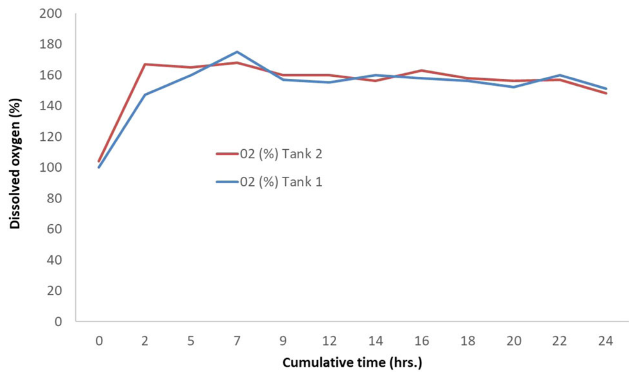


**FIGURE 11** Dissolved oxygen saturation rate in two tanks where two captive bred *Carcharhinus melanopterus* (4.5 and 4.8 kg) were moved from Oberhausen (Germany) to Lisbon (Portugal) over 24 h

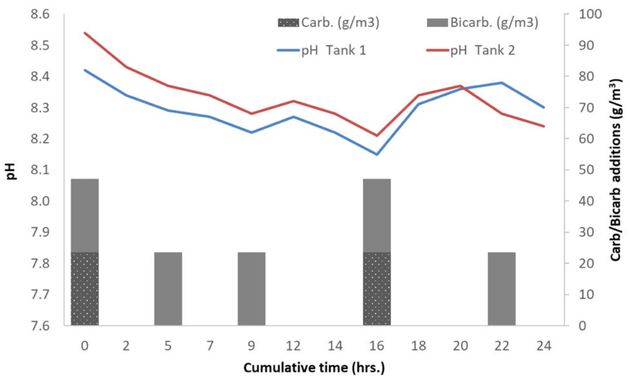


**FIGURE 12** pH in two tanks where two captive bred *Carcharhinus melanopterus* (4.5 and 4.8 kg) were moved from Oberhausen (Germany) to Lisbon (Portugal) over 24 h

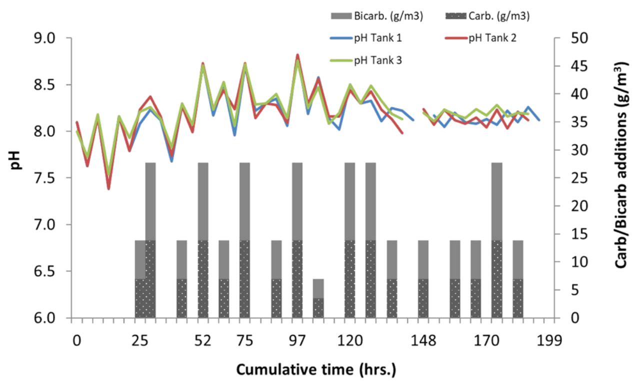


**FIGURE 13** pH in all three tanks loaded with 616 *Trachurus picturatus* (each), which travelled from Horta (Azores—Portugal) to Stralsund (Germany) over 200 h (8 days), including the addition of pH buffers, which were identical in all three tanks [Color figure can be viewed at wileyonlinelibrary.com]

pH was kept above 8.1 in both tanks, through multiple additions of buffers as displayed in Figure 12.

The *S. colias* which travelled inside plastic bags by air from Horta to Lisbon, then Frankfurt, then Tel Aviv, then by road to Jerusalem, arrived with a dissolved oxygen saturation rate above 200%, and pH between 6.6 and 6.8 among all the bags that were moved. As mentioned earlier, no mortalities occurred.

The large group of fish which travelled from Horta to Königswinter and Stralsund (Germany), displayed oscillating pH during the initial segment of the trip, which corresponded to the sailing by sea from Horta to Lisbon, over 5 days (Figure 13). During this segment of the journey, daily water changes were made with the surrounding seawater, which allowed for a raise in pH after the daily feeding of all animals, which caused drops. During each overnight period, safety regulations prohibited access to the cargo deck, so 50%–100% of pH buffers were used as a precautionary measure. Once on shore, the road segment of the trip ensued, and no more feeding occurred, which explains the more stable pH pattern in all three tanks.

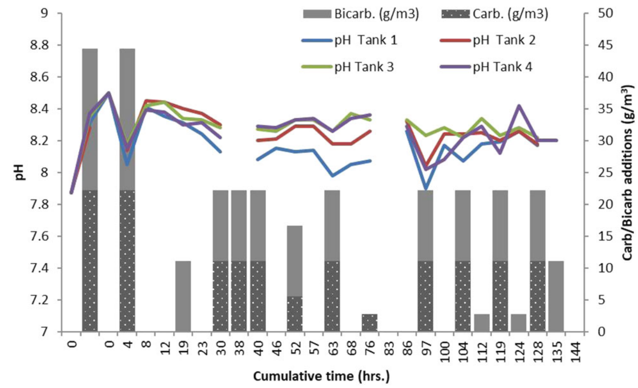


**FIGURE 14** pH in all four tanks loaded with 4 *Rhinoptera marmoratus* (tank 3 with only three animals), which travelled from Istanbul (Turkey) to Madrid (Spain) over 144 h (6 days), including the addition of pH buffers, which were identical in all four tanks [Color figure can be viewed at wileyonlinelibrary.com]

The following transport of a large number of animals, in July 2018, saw multiple additions and removals of animals, but its bioload peaked in La Ciotat, when 33 *P. americanus* were added to a tank already loaded with 4 *B. capriscus*, 1 *M. fusca*, 1 *S. notata*, 2 *M. squinado* and 2 *D. violacea*. The addition of the *Polyprion* (1 individual weighing 3 kg and the remaining 32 weighing 0.8 kg each), brought the overall bioload of the tank to 19.8 kg/m$^3$, but that diminished over the following days, as animals were dropped off in Berlin and Stralsund (Germany), Rotterdam (Netherlands) and Boulogne-sur-mer (France). Still, the batch of 8 individuals that was subsequently moved to Edinburgh by air, were in transit, by road, for 96 h. Those same animals were later packed individually in 42 L of water and shipped by air to Edinburgh. As mentioned earlier, no losses occurred in any of the steps mentioned above.

The 15 *R. marmoratus* which travelled from Istanbul to Madrid over 144 h (6 days), had no filtration running between cumulative hours 86 and 96, due to an equipment failure immediately before boarding a ferry (from Igoumenitsa, Greece, to Brindisi, Italy), which did not allow for water quality checks. Still, pH held stable during those 10 h (Figure 14), as did oxygen (Figure 15). In the absence of filtration, oxygen was turned up to 3 L/min during the overnight ferry crossing, which explains for the 300+% saturation rates the morning after. Conversely, a second equipment failure at approximately 116 cumulative hours, caused the blower unit to stop, which is why dissolved oxygen plummeted to approximately 50% in all tanks (Figure 15).

Tank 3 had no protein skimmer, which was due to yet another equipment failure and the animals were therefore loaded 4 in each of the remaining tanks and only three in tank 3. It is interesting to note, however, how water quality results in tank 3 were quite comparable to those from the remaining tanks.

Figure 16 depicts pH in all 4 tanks loaded with 300 *S. salar* smolts (each), that travelled from Sveio (Ireland) to Aveiro (Portugal) over 72 h. It also shows the additions of pH buffers, which were identical in all four tanks during each water quality check. Note how stable it

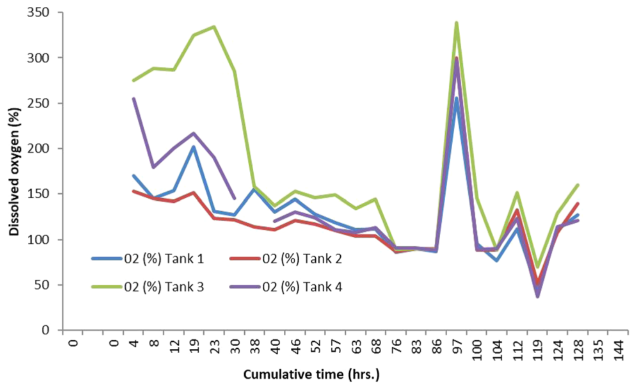


**FIGURE 15** Oxygen saturation rate in all four tanks loaded with 4 *Rhinoptera marmoratus* (tank 3 with only three animals), which travelled from Istanbul (Turkey) to Madrid (Spain) over 144 h (6 days) [Color figure can be viewed at wileyonlinelibrary.com]

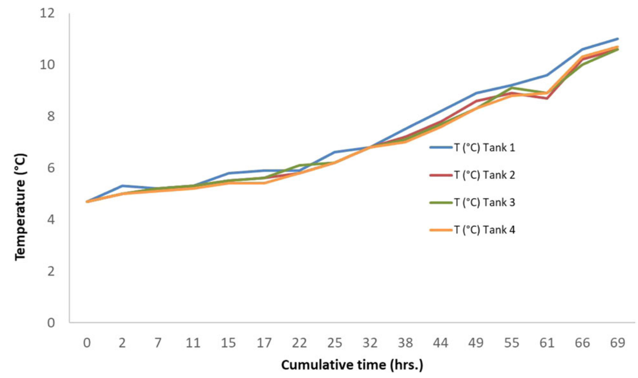


**FIGURE 17** Temperature in all four tanks loaded with 300 *Salmo salar* smolts (each), which travelled from Sveio (Norway) to Aveiro (Portugal) over 72 h (3 days). The objective was to arrive in Aveiro at 11°C

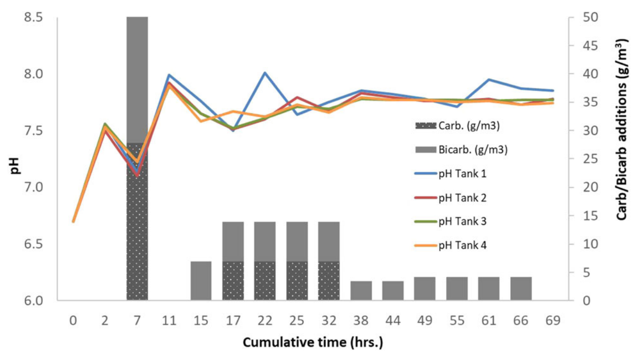


**FIGURE 16** pH in all four tanks loaded with 300 *Salmo salar* smolts (each), which travelled from Sveio (Norway) to Aveiro (Portugal) over 72 h (3 days), including the addition of pH buffers, which were identical in all four tanks

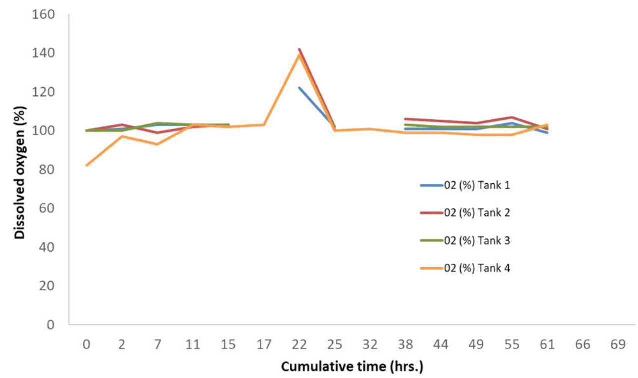


**FIGURE 18** Dissolved oxygen saturation rate in all four tanks loaded with 300 *Salmo salar* smolts (each), which travelled from Sveio (Norway) to Aveiro (Portugal) over 72 h (3 days). The flow rate was 1 L/min, with the exception of the noticeable spike at 20 h, when 2 L/min were used for a brief period

remained after 30 h. It is unclear as to why values in Tank 1 were slightly less stable than in the other 3, since the bioload, and filtration units were identical. One possible explanation could be the fact that Tank 1 was the farthest from the door and closest to the truck's heating unit, which created a lot of noise whenever operating. That disturbance is one possible explanation of less stable pH values. This hypothesis is somewhat corroborated by temperature values, with Tank 1 values again showing a pattern that is slightly different from the three remaining tanks (Figure 17).

Salinity was adjusted from 0 to 7 g/L upon loading of the animals, by adding sea salt from Tropical Marine Centre™. This addition, which had the objective of triggering the smoltification process, had an immediate positive effect in the foam fractionators, which began operating regularly upon the addition of salt.

Water temperature upon departure was 4.7°C. However, it was 11°C at the destination's holding facility, so temperature was allowed to raise throughout the trip (Figure 17), a process that occurred naturally, although closely monitored, and only required the truck's heating unit to be turned on towards the end of the trip.

With a flow rate of oxygen of 1 L/min in all tanks, the dissolved oxygen saturation rate remained close to 100% in all four tanks throughout the trip (Figure 18), the only exception being when there was a debit rate of 2 L/min in all tanks at approximately 20 h, which accounted for a sudden rise to 130+%. Since 100% was sufficient for the fish' wellbeing, the oxygen flow rate was lowered to 1 L/min once again, and so remained until the end of the transport.

The *S. salar* smolt transport from Letterfrack (Ireland) to Aveiro (Portugal) used the exact same equipment (Figure 19) and pH buffering philosophy highlighted before, which is why pH had almost identical values to the transport done from Sveio to the same destination (Figure 20). The only noticeable difference was the fact that the Norwegian animals were 200 g each, while the Irish lot were 100 g, which accounted for a bioload of 16.7 kg/m$^3$ in March and 8.3 kg/m$^3$ in December. Similarly to the trip taken in March from Norway, the Irish trip in December also included the addition of salt to all four tanks, so that salinity was raised from 0 to 7 g/L. Temperature was also allowed to increase naturally, and gradually, from

**FIGURE 19** General setup used in all four tanks loaded with 300 *Salmo salar* smolts (each), which travelled from Lettefrack (Ireland) to Aveiro (Portugal) over 76 h (3 days), which included an 18 h ferry crossing from Rosslare (Ireland) to Cherbourg (France) [Color figure can be viewed at wileyonlinelibrary.com]

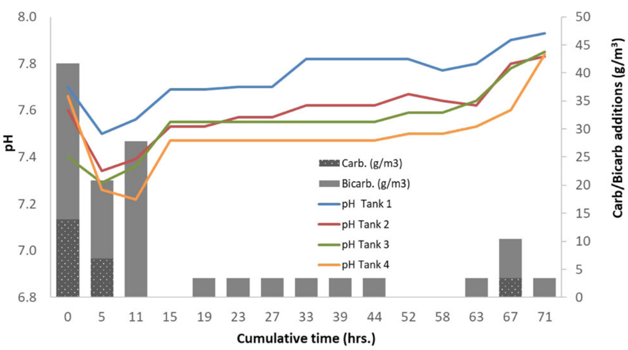


**FIGURE 20** pH in all four tanks loaded with 300 *Salmo salar* smolts (each), which travelled from Letterfrack (Ireland) to Aveiro (Portugal) over 76 h (3 days), including the addition of pH buffers, which were identical in all four tanks. During the last hours of the trip, an effort was made to match the pH of the destination facility (8.0), which was why the addition of pH buffers was intensified. Note the stability in pH between 15 and 40 h, particularly in tanks 3 and 4

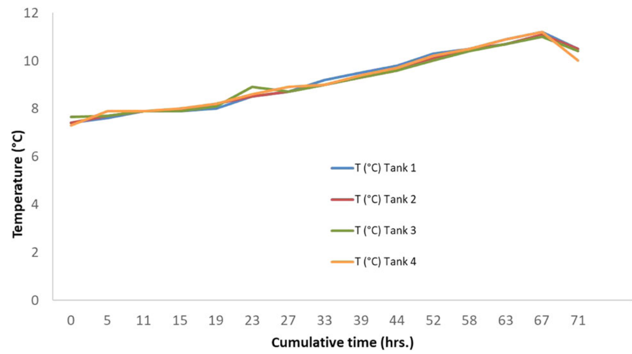


**FIGURE 21** Temperature in all four tanks loaded with 300 *Salmo salar* smolts (each), which travelled from Letterfrack (Galway) to Aveiro (Portugal) over 76 h (3 days). The objective was to arrive in Aveiro at 9°C [Color figure can be viewed at wileyonlinelibrary.com]

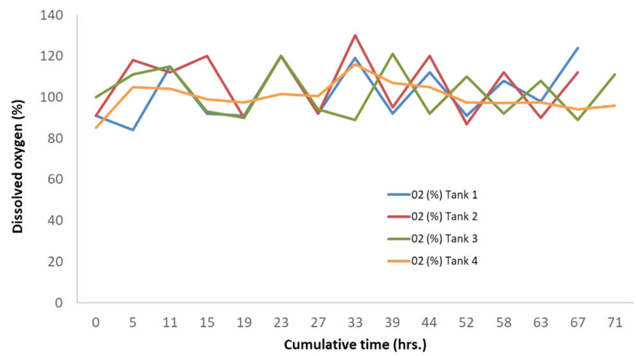


**FIGURE 22** Dissolved oxygen saturation rate in all four tanks loaded with 300 *Salmo salar* smolts (each), which travelled from Letterfrack (Galway) to Aveiro (Portugal) over 76 h (3 days)

7.4°C until 10°C, which was the value on arrival (Figure 21). This was slightly overshot, which prompted the team to turn on the cooling unit during the last 2 h of the trip, to ensure temperature was precisely 10°C on arrival. The dissolved oxygen saturation rate remained close to 100% throughout the trip, with small fluctuations (Figure 22).

The *S. scombrus* which travelled from Olhão (Portugal) to València (Spain) by road used the exact same equipment highlighted in all previous road transports, the only exception being the bioload, which was unusually high for this species. One tank carried 1006 fish, while another carried 891, and the third 833, which yields an average load of 910 fish per tank and, at 75 g each, a mean bioload of 19.0 kg/m$^3$. Despite these numbers, there were no noticeable differences between this 14 h trip from other previous similar transports (Figure 23).

The 600 *S. pilchardus* which travelled from La Ciotat (France) to Olhão (Portugal) over 19 h (Figure 24) also displayed water quality figures comparable to previously reported transports, with pH remaining stable around 8.1 through regular buffering with sodium bicarbonate and sodium carbonate, and ammonia remaining at 0 mg/L through the regular use of ATM Triage™ every 4 h.

**FIGURE 23** 2730 *Scomber scombrus* arrived to L'Oceanogràfic in València (Spain), after 14 h on the road from Olhão (Portugal). As in all cases reported earlier, no losses occurred

The 100 *S. scombrus* which travelled by air from Lisbon to Toronto inside a sealed 500 L tank, showed a dissolved oxygen saturation rate of 35% upon opening (Figure 25), after the flight and ground handling procedures both in Lisbon and Toronto, which totalled 14 h. An 8 h drive then followed to Biodôme Montréal, during which oxygen and multiple of the aforementioned doses of pH buffers and

**FIGURE 24** 600 *Sardina pilchardus* which travelled inside a 1600 L tank from La Ciotat (France) to Olhão (Portugal) over 19 h. Note the fully operational foam fractionator on the left side of the tank [Color figure can be viewed at wileyonlinelibrary.com]


**FIGURE 25** Dissolved oxygen saturation rates of 100 *Scomber scombrus* which travelled from Peniche (Portugal) to Montreal (Canada), via Toronto, inside a 500 L sealed tank, with no filtration during the 14 h of ground handling services at both airports and flight per-se. Left values on departure, immediately before the tank was sealed in Lisbon, and right values on arrival at Toronto, immediately after the tank was opened for the first time in 14 h. No losses occurred and oxygen plus pH buffers, and ammonia quencher were added immediately after

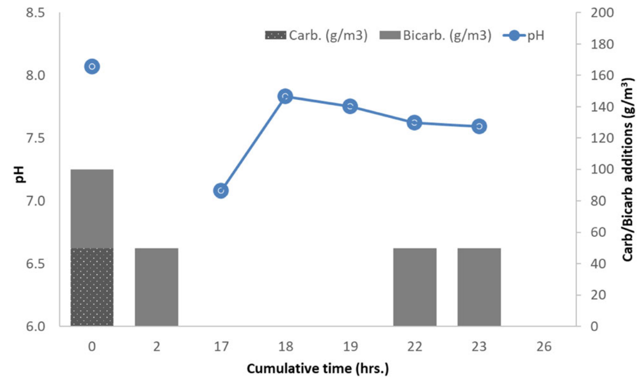


**FIGURE 26** pH of 100 *Scomber scombrus* which travelled from Peniche (Portugal) to Montreal (Canada) inside a 500 L sealed tank, with no filtration during the 14 h of ground handling services at both airports and flight per-se. New water was added at cumulative hours 17, 19 and 23, which accounts for the raise in pH despite no buffers being added [Color figure can be viewed at wileyonlinelibrary.com]

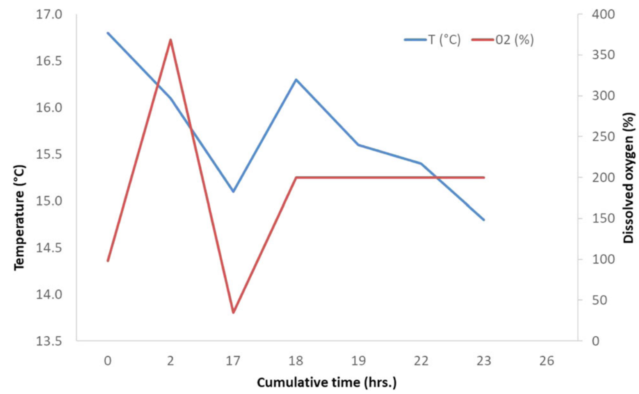


**FIGURE 27** Temperature and dissolved oxygen saturation rates of 100 *Scomber scombrus* which travelled from Peniche (Portugal) to Montreal (Canada) inside a 500 L sealed tank, with no filtration during the 14 h of ground handling services at both airports and flight per-se. The spike of 368% at 2 h was before the tank being sealed, while the low of 35.9% at 17 h was when opening the tank in Toronto

ammonia quencher were added, which allowed for pH to remain above 7.5 (Figure 26). Three loads of 300, 100 and 150 L of water at 20°C (first two) and 23°C (third) were also added during two stops in transit, with the objective of arriving to the destination facility at 18°C. Holding temperature was no easy task, despite two additions of water at 20°C (300 L at cumulative hour 17 and 100 L at hour 23) and a third addition of 150 L at 23°C at hour 23 (Figure 27). This is because air temperature was −5°C and the cargo area of the transport van had no climate control.

While all 100 individuals arrived alive and well, only one exhibited a small wound near the caudal fin. That one animal died one week after arrival.

A total of 5 *S. dumerili* and 29 *S. rivoliana* were moved in four of the 2.4 m wide tanks depicted in all previous road and water transports. The animals were packed in the tanks for two days before shipping by water between the Azorean islands of Faial and São Miguel. This first segment of the trip was done without any accompanying persons, in light of restrictions imposed by the shipping company, due to the Covid-19 pandemic. During this time, temperature, ammonia and pH were monitored remotely with a Seneye™ sensor (Figure 28), while a camera allowed to inspect that all equipment was functioning. With a mean weight of 1.6 kg each, the 34 animals were packed at approximately 9 fish per tank, yielding a bioload of 3.8 kg/m$^3$. In this case, the pH buffer used was ATM Alka-Haul™ at a concentration of approximately 22 g/m$^3$, and ATM Triage™ at 40 g/m$^3$ (Figure 29).

Once the first batch of 13 animals was delivered in São Miguel, the remaining 21 were split amongst the four tanks, which lowered the bioload to 2.3 kg/m$^3$. With one full day of operations in São Miguel, the remaining segment of the trip, by water, took an additional 4 days, after which 16 h by road followed from the port of Leixões (north of Portugal) to the IPMA research facility in Olhão (south of Portugal).

Once the remaining 21 *Seriola rivoliana* were delivered, the tanks were drained and immediately filled with local water and captive bred


**FIGURE 28** 5 *Seriola dumerili* and 29 *Seriola rivoliana* were moved from Faial island (Azores—Portugal) to São Miguel (Azores—Portugal) and to Olhão (mainland Portugal), after 1 + 4 days by sea. The first segment of the trip (between islands) was done without an escorting person, due to Covid-19 restrictions. A small camera and router allowed for continuous monitoring of the equipment, while a Seneye™ sensor allowed to constantly monitor temperature and pH [Color figure can be viewed at wileyonlinelibrary.com]

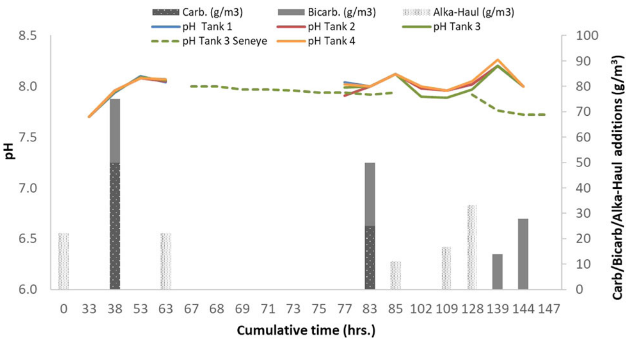


**FIGURE 29** pH in all four tanks loaded with 5 *Seriola dumerili* and 29 *Seriola rivoliana*, which travelled from Faial island (Azores—Portugal) to São Miguel (Azores—Portugal) and later to Olhão (mainland Portugal), after 1 + 4 days by sea, including the addition of pH buffers, which were identical in all 4 tanks. This trip included the addition of ATM Alka-Haul™ as a pH buffer as well. Note the interval when only data from the Seneye probe was available [Color figure can be viewed at wileyonlinelibrary.com]

*A. regius*. 200 animals of 200 g each were packed in Tank 4 (with a bioload of 11.1 kg/m$^3$, Tab. 1), while 51 others, with a mean weight of 10 kg, were packed in Tanks 1, 2 and 3, therefore with a bioload of 47.2 kg/m$^3$ (Table 1) each. As before, all animals arrived alive and well to the IRTA research facility in Sant Carles de la Ràpita (Spain), after 15 h on the road.

It is interesting to note that, at 11 h of transport, while Tank 1 received 55.6 g/m$^3$ of sodium bicarbonate—which is similar to what all tanks received at 5 h—Tanks 2, 3 and 4 were dosed with 22.2 g/m$^3$ of Alka-Haul instead (Figure 30). The difference in behaviour of pH is quite noticeable, with sodium bicarbonate having no effect in a continued decrease (in Tank 1), while Alka-Haul drove pH to rise in Tanks 2, 3 and 4.

Temperature (Figure 31) is also worthy of mentioning, since there was an unexpected difference between Tank 4 and the remaining three, which had very similar values throughout the trip. This is most likely a result of the position of Tank 4 inside the 40 feet shipping container in which the *Seriola* travelled from the Azores and

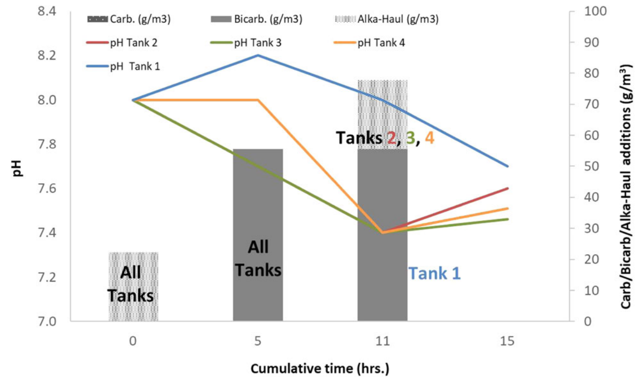


**FIGURE 30** Captive bred 200 small (200 g) *Argyrosomus regius* (Tank 4) and 51 large (10 kg) *A. regius* were moved from Olhão (Portugal) to Sant Carles de la Ràpita (Spain) over 15 h by road, including pH buffering. Note that carbonate was not used, but ATM Alka-Haul™ was used instead [Color figure can be viewed at wileyonlinelibrary.com]

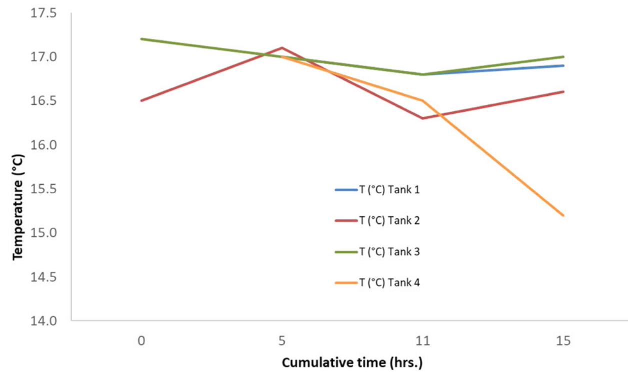


**FIGURE 31** Temperature in tanks with 200 small (200 g) *Argyrosomus regius* (Tank 4) and 51 large (10 kg) *A. regius* were moved from Olhão (Portugal) to Sant Carles de la Ràpita (Spain) over 15 h by road. Tank 4 was positioned closest to the door of the container, which seems to have had a significant impact on temperature [Color figure can be viewed at wileyonlinelibrary.com]

then the *Argyrosomus* travelled to Spain. Tank 4 was the one closest to the door and this seemed to have a significant impact on temperature. Dissolved oxygen remained above 100% throughout the trip (Figure 32).

The 5 *P. americanus* transport from La Ciotat (France) to Galway (Ireland) had to be split in two stages for operational reasons, which forced the animals to spend approximately two months at L'Ocearium du Croisic, where they arrived 14 h after departure. Once the final leg began (in Le Croisic), total transport time until Galway was 34 h, which included an 18 h ferry crossing from Cherbourg to Rosslare. This transport was identical to all previous ones which used a 1.9 m tank, mentioned earlier, but the filtration was upgraded with a chiller unit and arranged in a more functional fashion, to maximize usage of space inside the van (Figure 33). This transport also included an initial dose of 25 g/m$^3$ of ATM Alka-Haul™ (Figure 34).

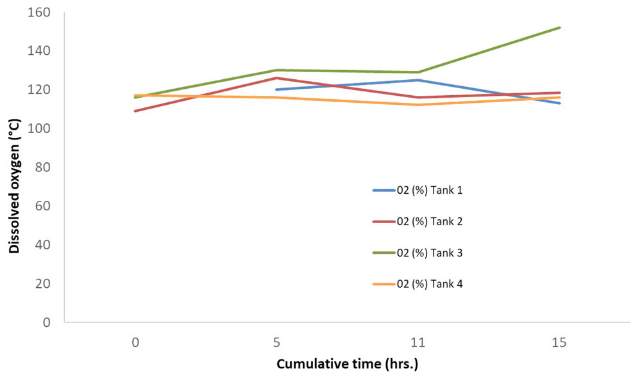


**FIGURE 32** Dissolved oxygen saturation rate in tanks with 200 small (200 g) *Argyrosomus regius* (Tank 4) and 51 large (10 kg) *A. regius* were moved from Olhão (Portugal) to Sant Carles de la Ràpita (Spain) over 15 h by road. Tank 4 was positioned closest to the door of the container, which seems to have had a significant impact on temperature

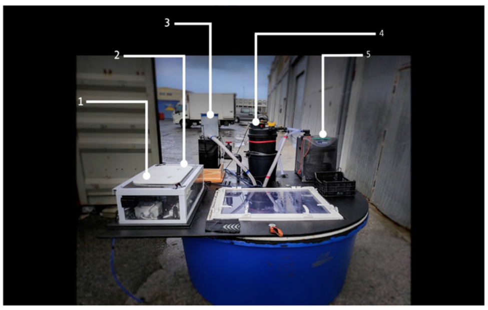


**FIGURE 33** Filtration mounting on lid of 1.9 m wide tank during transport of 5 *Polyprion americanus* from Le Croisic (France) to Galway (Ireland), and back with 600 *Salmo salar* smolts to Vila Real (Portugal)

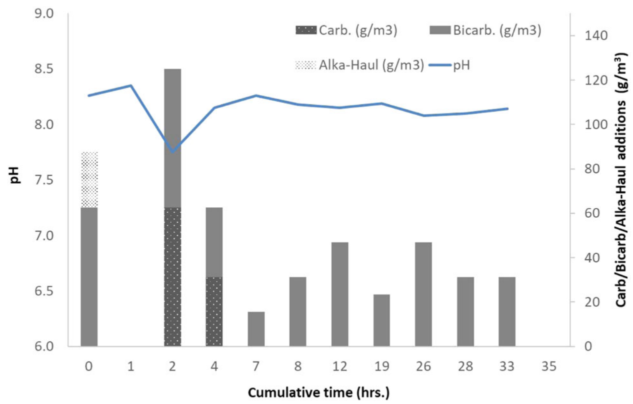


**FIGURE 34** pH of 5 *Polyprion americanus* which travelled from Le Croisic (France) to Galway (Ireland) over 35 h, including additions of pH buffers

Caption: 1—sealed box for inverter unit and circuit breaker, accessible through nautical hatch; 2—blower; 3—foam fractionator; 4—mechanical foam filter, with activated carbon and UV; 5—chiller unit; operational volume: 1600 L.

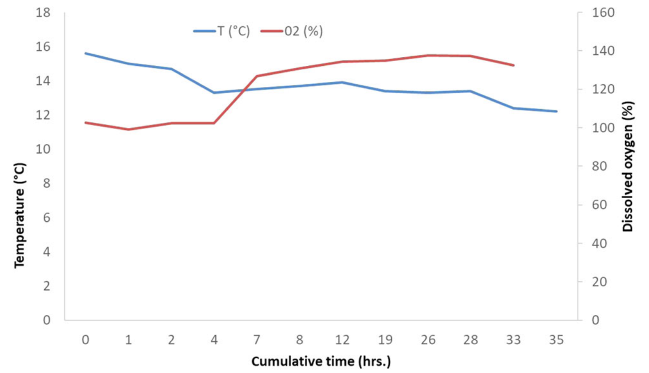


**FIGURE 35** Temperature and dissolved oxygen saturation rate of 5 *Polyprion americanus* which travelled from Le Croisic (France) to Galway (Ireland)

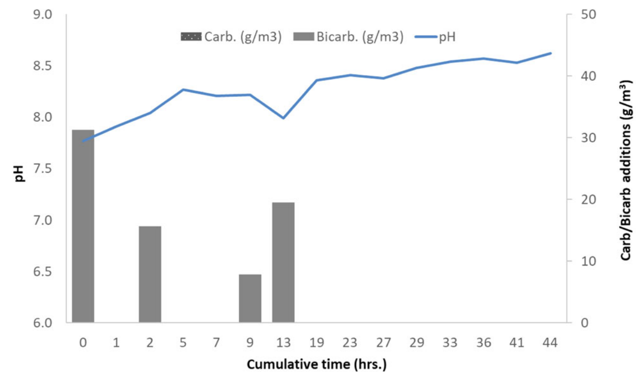


**FIGURE 36** pH of 600 *Salmo salar* which travelled from Galway (Ireland) to Vila Real (Portugal) over 46 h, including additions of pH buffers. Note that, after addition at 13 h, no further additions were made and still pH continued to climb

While the transport of the 5 *P. americanus* had 'standard' water quality parameters (Figures 34 and 35), the return with 600 *S. salar* in 0 g/L salinity had an unexpected effect on the pH probe, which consecutively showed growing values of pH, despite the fact that no buffers were added after 13 h (Figure 36). Still, the fish' appearance and calm behaviour did not suggest any indication of problem, and they were indeed safely delivered after 46 h in transit. Because this was a freshwater transport, no ATM Alka-Haul$^{TM}$ was used, since its effect on freshwater was unknown at the time. Temperature dropped during the initial phase of the trip, particularly during the ferry crossing from Rosslare to Cherbourg, because the deck where the van travelled was open (Figure 37). Dissolved oxygen remained above 100%, with only one spike that corresponded to a flow of 3 L/min, which was later cut back down to 2 L/min.

The *T. picturatus* that were flown to Aquarium of the Boardwalk, in Branson, MO, USA, were packed six per bag, in 21 L of water and similar volume of pure oxygen. As noted in previous plastic bags shipments, 100 mg/m$^3$ of sodium bicarbonate and a similar amount of sodium carbonate were added during packing,

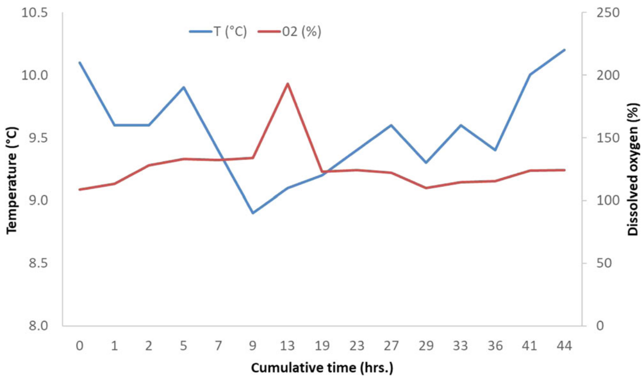


**FIGURE 37** Temperature and dissolved oxygen saturation rate of 600 *Salmo salar* which travelled from Galway (Ireland) to Vila Real (Portugal) over 46 h. The spike in oxygen at 13 h was due to an increase in flow from 2 to 3 L/min, due to the boarding of a ferry during the previous check

as did the also aforementioned 64 g/m$^3$ of ATM Triage™. After one flight from Horta to Lisbon and then a direct flight to Chicago plus import customs procedures and driving to Branson, 252 fish arrived after 36 h, with a temperature of 16.6°C, pH of 7.5, ammonia at 0.5 mg/L and four dead individuals, which were the only losses in all transports here described.

## 5 | DISCUSSION

With no significant losses on all transports listed in Table 1 (the only exception being the 4 *T. picturatus* lost in a group of 252 shipped by air and road from Horta [Azores, Portugal] to Branson [MO, USA]), the authors feel confident about the transport methods described. Additionally, lengths of transports ranged from 14 to 200 h (8 days), and bioloads from 3.8 to 76.9 kg/m$^3$, and still that did not have an adverse effect on survivorship, which the authors credit to the following main causes:

1) Pre-buffering the water with sodium bicarbonate and sodium carbonate at 50 g/m$^3$ (each), and/or ATM Alka-Haul™ at 25 g/m$^3$, and applying additional (partial or full) doses throughout each transport, whenever the transport tanks were accessible.
2) Pre-quenching ammonia with ATM Triage™ at 64 g/m$^3$, and applying additional (partial or full) doses throughout each transport, whenever the transport tanks were accessible.
3) Keeping the dissolved oxygen saturation rate above 100%, ideally above 150%.
4) Keeping temperature on the lower limit of each species' tolerance range.
5) Using foam fractionators to effectively eliminate organic matter from the water.
6) Using pure sine wave inverters, which allows for a steady supply of electrical current throughout the transport.

While the pH buffering protocol yielded relatively stable pH values throughout all trips where buffering occurred enroute, there is a sharp difference between the earlier trips—such as the one from Oberhausen to Lisbon (September 2017, Figure 12), to more recent ones, such as the ones from Sveio and Galway to Aveiro (March and December 2019, Figures 16 and 20). The difference lies in the fact that, in the earlier trips, a 'corrective' philosophy was adopted, whereby buffers were used whenever a drop in pH was observed; in more recent transports, however, a 'preventive' approach was adopted, whereby buffers are used even if there is no drop in pH, because one assumes that pH would drop within the next few hours, if no buffers were used. This translated into somewhat erratic pH values—although within a narrow window—during the early transports, and a strikingly 'flat' pH line in more recent ones (e.g. Figure 16).

The use of sodium bicarbonate proved to provide better results than sodium carbonate, which is why Figures 13, 16, 20, 26, 30 and 34 reveal a more abundant use of bicarbonate, instead of carbonate. This is easily understandable if we remember the classic equation of pH buffering in the sea, whereby carbon dioxide and water react into carbonic acid, which will then split into bicarbonate, by releasing a proton, then becoming carbonate by releasing a second proton.

$$CO_2 + H_2O <=> H_2CO_3 <=> H^+ + HCO_3^- <=> 2H^+ + CO_3^{2-}.$$

This means that the addition of carbonate will have a greater impact on pH, shifting the equilibrium of the latter equation to the left. Bicarbonate will do the same, but at a lower rate. Traditionally, it is considered that carbonate will 'raise' pH, while bicarbonate assists in 'maintaining' it stable, which is consistent to what we have observed in all transports, and the reason why bicarbonate is preferred to carbonate.

In more recent transports ATM Alka-Haul™ was used, and it proved quite helpful in buffering pH, almost eliminating the use of carbonate altogether. While further study and transports are required for a full grasp of its potential, these initial trials are quite encouraging, particularly when Figure 30 is observed, whereby it is quite noticeable that sodium bicarbonate did not prevent a continued decline in pH, but ATM Alka-Haul™ reversed said declined into a raise.

The use of foam fractionators proved to be indispensable, which was particularly evident when the effluent was dumped during water quality checks. Longer trips, such as the 72+ h journeys from Sveio and Galway to Aveiro, saw no less than 100 L of extremely foul-smelling and murky water removed from each tank through the foam fractionators. One good way of empirically assessing the efficacy of this equipment is by imagining placing such 'dirty' water back into the transport tanks (!), which is of course inconceivable. It is therefore our firm belief that the use of foam fractionators is imperative in such long-term transports, whenever possible.

The use of voltage inverters also proved quite advantageous, mostly in the 'stability' of the filtration equipment's operation, particularly with foam fractionators, which had an annoying tendency of constantly demanding attention—and regulation of output flow—when 12 V DC batteries were the standard source of power. Indeed, as these batteries lost their power, so did the pumps driving water through the foam fractionators, which required constant fine-tuning on all water quality checks. The use of a steady supply of a 220 V AC current, allows for constant and regular flow, with minimum need for adjustments in the foam fractionators' outgoing flow. It is important to emphasise that 'pure-wave' inverters are preferred to regular ones, because the former are more efficient at keeping a steady current than the latter.

Temperature also played a decisive role in the success of these transports although, admittedly, the only 'scientific' way to prove this point would be to run identical transports with higher water temperatures, where we would most likely observe some mortality, due to an increase in metabolism. Indeed, temperature and metabolism have a close association, with multiple examples described for animals under human care (e.g., Correia, 1999). Despite the fact we lack corroboration, it is our belief that the success of these transports is largely (but certainly not exclusively) due to using the lowest possible temperature that does not fall outside each species' tolerance range.

In conclusion, we feel confident that these methods are adequate when moving fish over long periods of time, with bioloads as high as 76.9 kg/m$^3$, although we would not recommend that this bioload is used for all species, since we ourselves only used it on the *S. glanis*. But any value up to 20 kg/m$^3$ seems to fare perfectly with the equipment used and described before. As for future research, it is our wish to proceed with the widespread use of remote sensors, such as the ones provided by Seneye™ described earlier. These allow for constant monitoring, even if the attendants are not present, and facilitate the transfer of information to electronic platforms, so that data may later be worked upon with the use of spreadsheets and similar tools. It is also our wish to explore further use of ATM Alka-Haul™, since early results seem to indicate this substance's ability in holding pH steady for longer periods of time, in relation to sodium bicarbonate or sodium carbonate.

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## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## ETHICS STATEMENT

The animals mentioned in this manuscript were transported by the authors between approved Zoological institutions and, while they were not being used for scientific purposes per-se, all transports adhered to Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Furthermore, the authors are fully licenced to perform such activities, including collection from the wild, when applicable. Specifically:

- Transporter authorisation number PT6437R issued by the Portuguese General Directorate for Agriculture and Veterinary—www.dgav.pt
- Operator/Receptor number V90054B issued by the Portuguese General Directorate for Agriculture and Veterinary—www.dgav.pt
- Commercial operator number PT 07 074 OC issued by the Portuguese General Directorate for Agriculture and Veterinary—www.dgav.pt
- Most recent annual permit for collections of fish from the wild authorised by the Portuguese General Directorate of Marine Resources and Maritime Transport, reference number 21/2021/DRI/04-01-2021—www.dgrm.mm.gov.pt

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. All data was obtained by the authors during the transports described, and the full data set is available from the authors upon request.

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