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TECHNICAL REPORT

Long-Term Transportation, by Road and Air, of Chub Mackerel (*Scomber japonicus*) and Atlantic Bonito (*Sarda sarda*)

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During the second semester of 2009, three trips were made from Olhão (Southern Portugal) to Stralsund (Northern Germany) carrying 2.122 animals, which included multiple teleosts, elasmobranchs and invertebrates. This group included scombrids, such as 1.869 *Scomber japonicus* and 9 *Sarda sarda*, which are notoriously difficult to transport. However, multiple adaptations to transport regimes adopted regularly have allowed the authors to successfully move these animals by road and air over a total of up to 25 hr. Such adaptations included maintaining oxygen saturation rates at approximately 200%, and also the constant addition of AmQuel®, sodium bicarbonate, and sodium carbonate. Different formulations were used during the three trips, with the best results corresponding to 20/30/30 ppm of the three aforementioned chemicals, respectively. The authors suggest, however, that a modified formula of 20/40/40 ppm will allow for an even more stable pH on future trips. Zoo Biol 30:459–472, 2010. © 2010 Wiley-Liss, Inc.

Keywords: husbandry; aquariology; captive; AmQuel®; scombrid; pH buffer

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INTRODUCTION

In 2000, Charles Farwell presented a literature review on biological data from multiple pelagic species and the way this information had been used to increase success while keeping captive scombrids. This work was presented at the V International Aquarium Congress and was published in the event's proceedings 1 year later [Farwell, 2001a]. In his work, Farwell reported on numerous studies and also summarized how data on respiration rates for different species of scombrid fish of various sizes and at different temperature regimes were useful in system design. This enabled the exhibit designer to calculate the total number of specimens to be displayed at a given time. The author also reported how individual growth rates of wild fish could be combined with long-term growth data of captive specimens to calculate the total biomass of the exhibit.

At the time the display and keeping of tunas (Family *Scombridae*) in public aquaria was a recent breakthrough, with no more than six aquaria in Japan and the Monterey Bay Aquarium in the United States successfully displaying scombrid fish in their exhibits. Ten years later, this scenario has not changed substantially, with little more than an additional half-dozen institutions displaying these elusive animals to their audiences. Amongst these is the Oceanário de Lisboa and its impressive 3.000 *Scomber japonicus* school swimming in the Global Ocean exhibit.

Those animals, however, were caught at a set-net located 200 miles to the South, with a road transport of little more than 4–5 hr between the temporary holding facility and the aquarium. The real challenge came when a request for a similar size school came from a brand new facility of the German Oceanographic Museum, the Ozeaneum, located in Stralsund, Northern Germany. Both road and air segments of the transport between Olhão and Stralsund added up to 25 hr transport time. This would require minute preparation and careful attention to detail as well as a careful revision of literature available on the subject.

In 2001, Barbara Block and Ernest Stevens edited the book *Tuna*: *Physiology*, *ecology*, *and evolution*, which featured a whole chapter dedicated to tunas in captivity, again authored by renowned scientist Charles Farwell [Farwell, 2001b]. In this chapter, Farwell provides ample detail on the keeping of tuna for both scientific and commercial purposes. But the author also provides valuable information on the collection, transport, and husbandry methods of tunas, with an emphasis on Bluefin tuna (*Thunnus thynnus*) kept at the Monterey Bay Aquarium. For years, this information has been the main source of knowledge for all those working with captive tuna and Farwell's protocols have been adapted by many, including the authors of this article, who began working with scombrids in 1997 while introducing both *S. japonicus* and *Sarda sarda* to the Oceanário de Lisboa. The aforementioned "4–5 hr" transports from Olhão to Lisboa certainly seem "easy" now, but were far from it in 1997–1998. And Farwell's multiple publications played a key role in devising a protocol for such transports.

Throughout the years, Farwell's information on metabolic rates, and general husbandry aspects, proved to be very helpful to the authors of this article when focusing on the long-term transport of scombrid fish, driving the same authors to focus their attention on the oxygen saturation and removal of nitrogenous waste factors, because these animals have unusually high metabolic rates. Farwell [2001a,b] cites multiple authors to substantiate this notion, but since that time more additional references on the subject of scombrid physiology have emerged, such as Marcinek et al. [2001], Nauen and Lauder [2002], Blank et al. [2007], and Clark et al. [2010].

Additionally, the Tuna Research and Conservation Center has been a source of extremely valuable information on scombrid biology, physiology, and ecology, with countless articles that have become references in their fields, such as Blank et al. [2004], Block et al. [2005], and Teo et al. [2007], to name a few of the most significant ones. This information was consolidated with previous results that involved the authors of this article, such as Smith [1992], Correia [2001], Young et al. [2002], Smith et al. [2004], and the more recent article by Correia et al. [2008].

In these latter articles, the authors provide an extensive background of the physiological aspects involved during long-term transport by road and air. These include the logistic measures that have to be taken into place to counteract such concerns. Additionally, ammonia excretion and pH are the traditional main points of concern during live marine animal transport as, during a transport, pH will gradually decrease while ammonia will increase as a result of carbon dioxide build-up and the release of nitrogenous waste and miscellaneous stress related metabolites, respectively. Both these tendencies need to be counteracted through the use of filtration and/or chemical supplements. The control of pH can be achieved by the use of buffering agents, such as the tribuffer described by McFarland and Norris [1958], common baking soda (i.e. sodium bicarbonate—$NaHCO_3$) or soda ash (i.e. sodium carbonate—Na_2CO_3). Ammonia (NH_3 and NH_4^+) may be removed with the assistance of quenching agents, such as AmQuel® ($HOCH_2SO_3$) (Novalek, Inc., Hayward, CA), which binds to ammonia and transforms it into nontoxic aminomethanesulfonate ($H_2NCH_2SO_3^-$) and water. This substance has been successfully used in the transport of marine species for many years, specifically elasmobranchs [Visser, 1996; Young et al., 2002; Correia et al., 2008]. Oxygen dissolved in water will also decrease during transport as a direct result of respiration. This is easily counteracted by supplying oxygen through the use of an airstone connected to a cylinder of compressed medical grade oxygen. In the specific case of scombrid transport, however, concerns with ammonia removal and pH buffering were superseded by two additional variables: space and oxygen consumption.

The aforementioned literature on scombrid physiology frequently mentions the unusually high metabolic rates these animals exhibit. It was, therefore, paramount to minimize their need to exert excessive exercise, which implied using larger than usual transport tanks. The very same physiological trait also implied providing larger than usual oxygen saturation rates in the water and, in parallel, lower than usual animal bioloads in the transport tanks.

Regarding collections, the Southern coast of Portugal (a region known as Algarve) is a passageway for animals swimming in and out of the Mediterranean Sea during their migratory patterns. The commercial fishing company Tunipex S.A. established a set-net in this location. This set-net is the only one of its kind in Portuguese waters and, as partners of Flying Sharks, has been a source of live marine animals for public aquaria worldwide, such as L'Oceanogràfic in Valencia/Spain (www.cac.es/oceanografic); Georgia Aquarium in Atlanta/U.S.A. (www.georgiaaquarium.org); Virginia Aquarium & Marine Science Center in Virginia Beach/U.S.A. (www.virginiaaquarium.com); Atlantis—the Palm in Dubai/U.A.E. (www.atlantisthepalm.com); Tokyo Sea Life Park in Tokyo/Japan (www.tokyo-zoo.net); the Ozeaneum in Stralsund/Germany (www.ozeaneum.de); and the Kattegatcentre in Grenaa/Denmark (www.kattegatcentret.dk).

Set-nets are a preferred fishing method for public aquaria, as fish are allowed to swim freely before capture and no invasive steps are involved in the removal from the ocean (Fig. 1). This means that neither hooks nor any other skin perforating gear is ever used. The set-net operating off the Algarve shore captures a diverse array of both teleost and elasmobranch species (e.g. *Auxis rochei*, *Euthynnus alleteratus*, *Katsuwonus pelamis*, *S. sarda*, *T. thynnus*, *Xiphias gladius*, *Cetorhinus maximus*, *Isurus oxyrinchus*, *Prionace glauca*, *Mobula mobular*, *Manta birostris*, and many others), with the unique advantage of covering a wide range of sizes, especially with elasmobranchs, from newborns to fully grown. It was, therefore, no surprise that most of the institutions named above demonstrated an interest on acquiring scombrids should they enter the set-net.

MATERIALS AND METHODS

All animals were collected by Tunipex's and Flying Sharks' staff over multiple days between May and November 2009. Once caught in the set-net, the animals were removed from the ocean using nonabrasive vinyl stretchers or plastic bags (Fig. 1), and transported to land inside a 1.6 m diameter round polyethylene vat filled 1.0 m high with seawater. The trip to shore took approximately 1 hr, during which oxygen was added to the water from a compressed oxygen cylinder and airstone. Dissolved oxygen was maintained above 150% saturation.

Once reaching shore, *S. japonicus* and *S. sarda* were immediately transferred to one 10.0 m diameter $\times$ 1.8 m high round fiberglass tank. This staging tank is on a flow-through system and has no temperature control. Introduction was, therefore, preceded by a quick acclimation period (typically 10–15 min). Filtration of the staging tank consists of one rapid 2 m^3 sand filter for mechanical filtration and one elevated tower, loaded with standard polyethylene "bioballs" for biological

Fig. 1. Collection of *Scomber japonicus* at the Tunipex set-net off Olhão (Portuguese Southern shore). Note plastic bags (1) used by collectors to haul fish from the ocean.

filtration. The system is kept on a semi flow-through regime with a daily turnover rate of approximately 100%. New water is pumped directly from the nearby shore as the staging facility is located in Olhão, deep inside the Ria Formosa lagoon complex.

One day after arrival, all animals were offered small (approximately 5–8 mm carapace length) frozen shrimp (*Palaemonetes varians*) by throwing it on the surface of the water. Food was offered at approximately 3% of the individuals' body weight per day. One to two days after food was offered, the animals would quickly swim toward the feeding place as soon as food was dropped in the water. Food was then offered 2 times per day. *S. japonicus* were estimated to weigh 0.1 kg each while *S. sarda* weighed 1.0 kg each.

About 1.869 *S. japonicus* and 9 *S. sarda* were transported to their final destination in three batches, usually 4–6 weeks after their respective arrival at the temporary holding facility. No treatments were done on these animals. Two days before traveling, all animals were fasted to decrease the amount of nitrogenous waste released during transport. Transports took place during the following dates and consisted on the routes described below, with additional details given in Table 1:

Seven hundred and fifty *S. japonicus* were moved in four tanks by road from Olhão to Lisbon on June 27, 2009, then flown to Leipzig and moved by road to Stralsund, with a total trip time of approximately 24 hr.

About 1.119 *S. japonicus* were moved in five tanks to Stralsund, using a similar route and identical trip time on August 1, 2009.

Nine *S. sarda* were moved in two tanks to Stralsund, using a similar route and identical trip time on November 28, 2009.

Table 1 includes other animals that were shipped during these three trips as well. However, this article focuses exclusively on those tanks carrying scombrids.

Transport tanks for the three missions described above followed a relatively simple concept previously used and described in detail by Correia [2001], Young et al. [2002], and Correia et al. [2008]. This simple method (Fig. 2) consists of polyethylene round containers with the following specifications:

About 1.6 m diameter $\times$ 1.0 m high for each group of approximately 208 *S. japonicus*. Transport tanks were filled 0.7 m high with 50% seawater from the temporary holding facility and 50% "clean" seawater purposefully brought from the ocean by Tunipex's staff. This yielded a volume of 1.3 m^3, which translated into a mean bioload of 16.0 kg/m^3 for *S. japonicus* (Table 1).

One similar tank carried four *S. sarda* by themselves with a bioload of 3.1 kg/m^3.

A second similar tank carried five *S. sarda* with additional other fish as well (see Table 1), yielding a bioload of 11.2 kg/m^3.

All tanks were equipped with one Jacuzzi® CFR 50 cartridge filter (Jacuzzi, Inc., Chino, CA) powered by a 12 V Rule® 2000 GPH bilge pump (ITT Industries, Inc., White Plains, NY) mounted on the lid; each cartridge consists of multiple laminated sheets of filter paper (45 μm) with the addition of one bag of activated carbon in the center.

All tanks were sealed with a fiberglass reinforced 15 mm thick wooden lid that was bolted to the rim, ensuring it was leak proof. This was particularly important while moving tanks by air and was the object of an official inspection by the Portuguese Institute of Welding and Quality (www.isq.pt). During such inspection, the tank was filled to the top, closed, sealed and pressurized to 0.1 bar. No leaks were detected. After this test, one tank was shaken vigorously. No leaks were detected

TABLE 1. Bioloads During Transport by Road and Air of *Scomber japonicus* and *Sarda sarda* from Olhão (Portuguese Southern shore) to Stralsund (German Northern shore)

Date	Species	♯ind.	Biomass (kg)	Bioload (kg/m^3)
Jun 27, 09	*Scomber japonicus*	212	21	16.3
Jun 27, 09	*Scomber japonicus*	220	22	16.9
Jun 27, 09	*Scomber japonicus*	152	15	11.7
Jun 27, 09	*Scomber japonicus*	166	17	12.8
Aug 1, 09	*Scomber japonicus*	224	22	17.2
Aug 1, 09	*Scomber japonicus*	210	21	16.2
Aug 1, 09	*Scomber japonicus*	224	22	17.2
Aug 1, 09	*Scomber japonicus*	224	22	17.2
Aug 1, 09	*Scomber japonicus*	237	24	18.2
Nov 28, 09*	*Sarda sarda*	5	15	11.2
Nov 28, 09	*Sarda sarda*	4	4	3.1
(*) Includes miscellaneous fish: 2 *Raja* sp., 1 *Torpedo* sp., 1 *Pagellus* sp., 1 *Seriola* sp., 1 *Spondyliosoma* sp., 3 *Octopus vulgaris*				
Total *S. japonicus*		**1.869**	**187**	–
Total *S. sarda*		**9**	**19**	–
Mean *S. japonicus*		**208**	**21**	**16.0**
Other specimens transported				
Jun 27, 09	*Boops boops*	1		
	Dasyatis pastinaca	1		
	Diplodus cervinus	39		
	Epinephelus marginatus	2		
	Lagocephalus lagocephalus	1		
	Mola mola	2		
	Mugil cephalus	5		
	Muraena helena	1		
	Octopus vulgaris	1		
	Pagrus pagrus	30		
	Palinurus elephas	7		
	Pteromylaeus bovinus	1		
	Raja clavata	1		
	Sarpa salpa	18		
	Scorpaena scrofa	10		
	Serranidae (Fam.)	1		
	Spondyliosoma cantharus	3		
	Torpedo torpedo	1		
	Trachurus trachurus	3		
Aug 1, 09	*Sarpa salpa*	25		
	Mugil cephalus	20		
	Raja undulata	2		
Nov 28, 09	*Sarpa salpa*	50		
	Dasyatis pastinaca	1		
	Octopus vulgaris	3		
	Palinurus elephas	10		
	Raja clavata	1		
	Sarda sarda	2		
	Seriola rivoliana	1		
	Torpedo torpedo	1		
Total teleosts		**214**		
Total elasmobranchs		**9**		
Total invertebrates		**21**		
Total animals shipped (scombrids included)		**2.122**		

The top segment of the table depicts one transport tank per line while other animals shipped are listed indiscriminately at the bottom of the table.

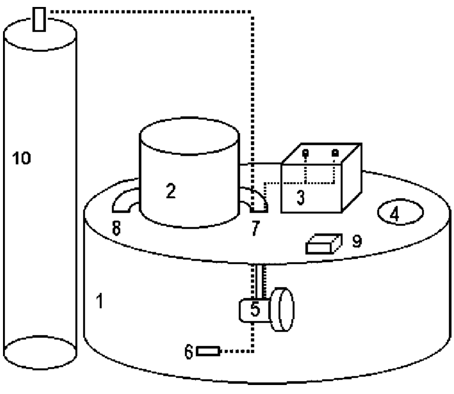

Fig. 2. General design of tank used for *Scomber japonicus* and *Sarda sarda* during transport by road and air. 1, Polyethylene transport tank (1.6 m diameter × 1.0 m high) with 15 mm thick fiberglass reinforced lid bolted to 30 mm gasket. 2, Filter unit. Contains one laminated paper cartridge and one mesh bag with activated carbon. 3, 12 V dry-cell sealed battery, wired to bilge pump. 4, Porthole, removable. 5, 12 V powered Rule® 2000 GPH bilge pump, pushes water up through filter. 6, Airstone fed by airline connected to pressurized oxygen cylinder. 7, Filter inlet, i.e. PVC elbow mounted through wooden lid, connected to bilge pump through 2.5 mm reinforced hose. 8, Filter outlet, i.e. PVC elbow mounted through wooden lid, returns filtered water above surface of water inside tank. 9, Small AA 1.5 V battery powered aeration unit, attached inside the lid. The airstone was connected to this unit whenever the use of oxygen from compressed cylinder was not permitted (e.g. inside aircrafts, during flight). 10, Pressurized oxygen cylinder, secured to pallet carrying tanks.

either. Each lid included a acrylic hatch (60 × 60 cm wide) that allowed for visual inspections of the animals and also their placement inside the tank.

All animals were collected from the temporary holding tanks and moved inside their respective transport tanks using transparent plastic bags. Handlers used latex gloves to prevent damage of the skin should any contact happen accidentally. While in transport, animals and equipment were checked approximately every hour. Checks included monitoring the animals, equipment, and water quality parameters, such as temperature and dissolved oxygen (using a hand held OxyGuard® Handy Oxygen probe®—OxyGuard Intl., Denmark), pH (using a hand held OxyGuard® Handy pH® probe), and ammonia (using a Palintest® Photometer 7000® photometer—Palintest, Tyne and Wear, UK).

Oxygen was typically supplied at a rate of 1–2 l/min and this flow was raised if dissolved oxygen dropped below 150% saturation. Ammonia (total ammonia nitrogen) test results higher than 0.25 ppm were immediately counteracted by adding AmQuel® to the water. Each dose of AmQuel® also contained a dose of sodium bicarbonate and sodium carbonate, as the use of AmQuel® is known to be associated with a decrease in pH. This "cocktail" of AmQuel® + sodium bicarbonate + sodium carbonate was calculated with the objective of quenching 1 ppm of ammonia in 1 m^3 of water, which was the approximate volume carried inside the tanks.

AmQuel® and pH buffering agents were dosed using the following proportions and concentrations during each transport:

June 27, 2009: 15 g AmQuel® + 15 g sodium bicarbonate + 30 g sodium carbonate (i.e. 11.5/11.5/23.1 ppm).

August 1, 2009: 40 g AmQuel® + 20 g sodium bicarbonate + 40 g sodium carbonate (i.e. 30.8/15.4/30.8 ppm).

November 28, 2009: 25 g AmQuel® + 40 g sodium bicarbonate + 40 g sodium carbonate (i.e. 19.2/30.8/30.8 ppm).

Dosing of AmQuel® and pH buffering agents was done separately for AmQuel® and bicarbonate + carbonate and consisted on dissolving small packets of preweighed chemicals (i.e. 15 and 30/30 g or 40 and 20/40 g or 25 and 40/40 g) in a 3 l container, shaking it vigorously and dropping the liquid contents directly on the surface of the transport tank water.

All three trips had similar routing and timings:

Loading in Olhão (Portugal): 0 hr transit.

Departure by road from Olhão: 2 hr transit.

Arrival at DHL cargo terminal in Lisbon (Portugal): 6 hr transit.

Departure by air from Lisbon on DHL's Boeing 757: 10 hr transit.

Landing in Leipzig (Germany): 13 hr transit.

Departure by road from Leipzig: 16 hr transit.

Arrival to Stralsund and begin acclimation: 22 hr transit.

Finish acclimation and introduction of animals to quarantine tanks: 24 hr transit.

Acclimation typically consisted of removing 50% of the water volume in each tank and then filling it with an equivalent volume of "new" water from the quarantine facility to where the fish were being transferred.

RESULTS

All 2.122 animals (i.e. 1.878 scombrids, 214 other teleosts, 9 elasmobranchs, and 21 invertebrates) arrived alive and well at their destination, except for 2 of the 9 *S. sarda*, which died approximately at 15 and 20 hr in transit. Subsequently, two more *S. sarda* perished during the 2 weeks after arrival. No *Scomber scombrus* died in that interval. Water quality results of all three transports are given in Figures 3–6.

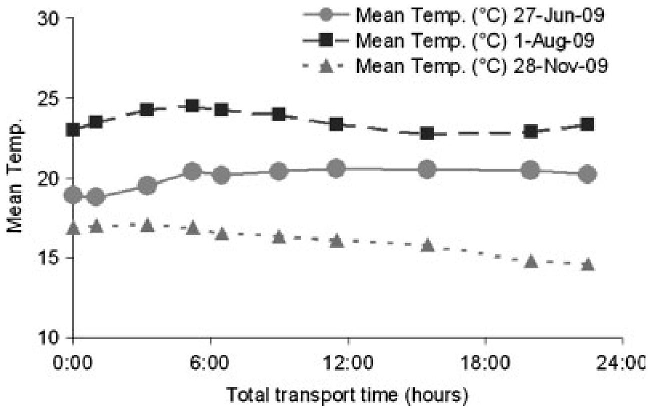

Fig. 3. Mean water temperature during transport by road and air of *Scomber japonicus* and *Sarda sarda* from Olhão (Portugal) to Stralsund (Germany) ($n = 4, 5, 2$ tanks during June 27, August 1, and November 28, respectively).

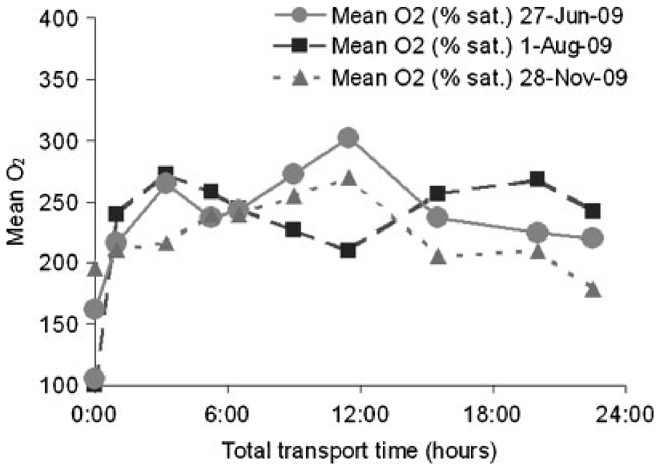

Fig. 4. Mean oxygen saturation rates during transport by road and air of *Scomber japonicus* and *Sarda sarda* from Olhão (Portugal) to Stralsund (Germany) ($n = 4, 5, 2$ tanks during June 27, August 1, and November 28, respectively).

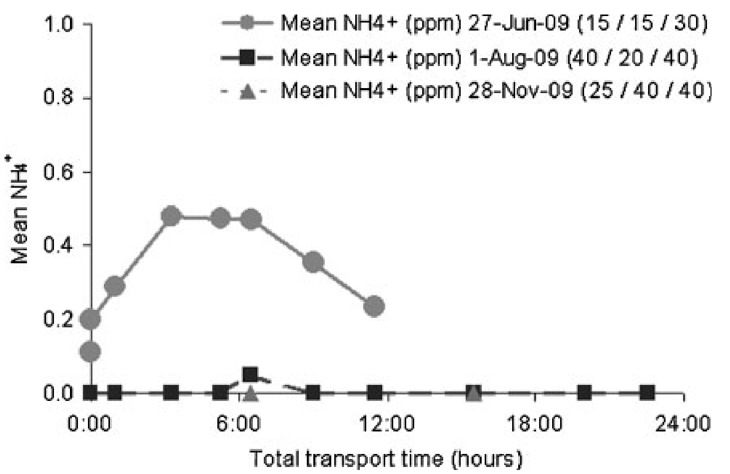

Fig. 5. Mean ammonia rates during transport by road and air of *Scomber japonicus* and *Sarda sarda* from Olhão (Portugal) to Stralsund (Germany) ($n = 4, 5, 2$ tanks during June 27, August 1, and November 28, respectively).

Water temperatures were quite different in all three transports (Fig. 3), with highest values in August (overall mean of 23.6°C), lowest in November (overall mean of 16.2°C), and mid values in June (overall mean of 20.0°C), as expected in a typically temperate climate, such as the one influencing Western Europe during these months. Temperatures remained relatively stable throughout the trips, despite the fact that no temperature control was applied. Only during the Lisbon to Leipzig flights were the aircrafts' cargo holds regulated to 15°C.

During the November 28 transport, there was a noticeable decrease in temperature throughout the trip (from 17.0 to 14.6°C), but this did not seem to have an adverse effect on the animals.

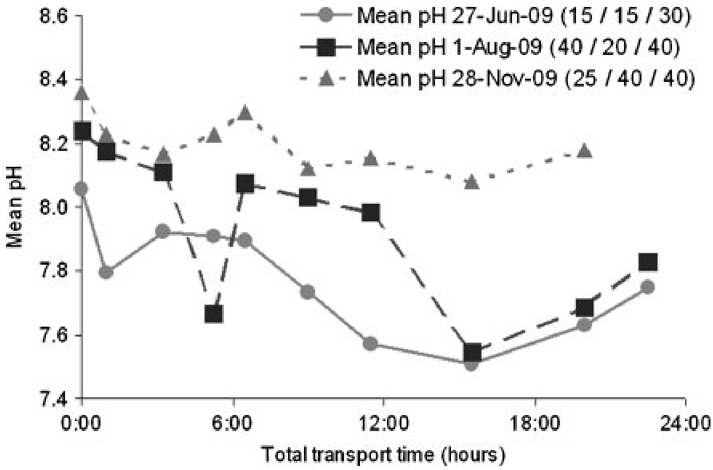

Fig. 6. Mean pH during transport by road and air of *Scomber japonicus* and *Sarda sarda* from Olhão (Portugal) to Stralsund (Germany) ($n = 4$, 5, 2 tanks during June 27, August 1, and November 28, respectively).

Oxygen saturation rates were maintained above 200% throughout all three transports (Fig. 4) by dosing oxygen addition from 1 to 3 l/min. Dosing was mostly kept at 2–3 l/min during the June and August transports, whereas dosing of only 1 l/min was sufficient during November to keep saturation rates high. This was most likely owing to the relatively higher water temperatures during June and August and, therefore, lower capacity for oxygen saturation.

Ammonia concentrations were not measured hourly as previous variables were, but showed relatively higher values during the first hours of the June trip, reaching a maximum of 0.48 ppm after 4 and 6 hr in transit (Fig. 5). These measurements were always followed by the addition of the "AmQuel+bicarb+carb cocktail," which had its lowest values in June, i.e. 15/15/30 g.

During the August trip, these values were increased to 40/20/40 g, with immediate and obvious positive results, as ammonia concentration never surpassed 0.05 ppm. After these results, 40 g of AmQuel seemed, therefore, unnecessarily high and were lowered to 25 g, with "cocktail" proportions of 25/40/40 g in November. This "recipe" yielded 0 ppm ammonia throughout the trip.

In total, there were eight "cocktail" additions during the June trip, with only the last one consisting solely on bicarbonate and carbonate. These additions were made approximately every 3 hr.

There were also eight "cocktail" additions during the August trip, with only one addition (half-way) consisting solely on bicarbonate and carbonate. Again, these additions were made approximately every 3 hr.

The November trip had the lowest bioloads of all and chemical additions were made separately for AmQuel® and bicarbonate plus carbonate. There were nine bicarbonate plus carbonate additions, made approximately every 2.5 hr. There were only three additions of AmQuel® in the tank with the five *S. sarda* and miscellaneous fish (at 0, 4 and 22 hr of transport time) and only one single addition of AmQuel® to the tank with the four *S. sarda* done at the very beginning of the trip.

Water pH dropped during all trips (Fig. 6), with decreasing values being more obvious during the June and August trips. The best pH results (i.e. with relatively lesser decreases with time) were obtained with the third "cocktail recipe" which, naturally, contained the highest dosing of buffering agents, i.e. 25/40/40 g. Still, the authors speculate that an even higher "recipe" of 25/50/50 g will most likely keep pH even more stable through future trips.

As expected, there was a strong correlation between ammonia and pH with bioload, with higher bioloads yielding higher ammonia concentration and lower pH. However, although this cause–effect relationship is fairly obvious during the June 27 trip, it becomes less obvious in the August 1 and November 28 trips, as "AmQuel® + bicarb + carb cocktail" concentrations used were higher. As such, the higher the "cocktail" concentration, the lesser is the influence caused by bioload in both ammonia and pH. This was particularly obvious during the November 1 trip, where the highest "cocktail" concentrations yielded lower ammonia concentration and higher pH throughout the trip.

Upon arrival *Sarda sarda* were kept in a round 6 m in diameter fibreglass 30 m^3 quarantine tank together with all the rays transported as well (i.e. 1 *Dasyatis pastinaca*, 1 *Pteromylaeus bovinus*, 1 *Raja clavata* and 1 *Torpedo torpedo*). During this initial period the following treatments were administered to the tank:

1. 1 ml of Catosal® (Bayer, Leverkusen, Germany) per 1,000 l of tank volume for 5 days in a row (30 November to 4 December 2009). Catosal® is an organic phosphorous and vitamin B12 tonic.
2. 20 ml of Trimetox® 240 (Veyx, Schwarzenborn, Germany) per 1,000 l of tank volume for 3 days in 2 day intervals (1, 4 and 6 December 2009). Trimetox® 240 is a broad-spectrum anti-microbial agent.
3. 0.5 ml of Trimetox® 240 (Veyx, Schwarzenborn, Germany) per 10 ml of water poured in food and soaked for 20 minutes during 13 days (from 14 to 26 December 2009).

After these treatments all skins abrasions, and visible signs of inflammation on all the *Sarda sarda* were healed completely.

DISCUSSION

The transport regime used for all 2.122 animals moved seemed adequate and yielded positive results, which means that there were no mortalities in transit. Of the 1.869 *S. japonicus* and 9 *S. sarda* moved only 2 of the latter died at 15 and 20 hr during the November 28 trip. These animals, however, were packed in a tank that presented less than ideal conditions, with an array of other fish and invertebrates (Table 1) packed together with the five *S. sarda*. The authors acknowledge that, in light of the well-known delicate nature of *S. sarda*, packing five animals with other non-scombrid teleosts was not ideal. However, the conditions were ideal for testing such a scenario.

This investigation allowed the conclusion that, although *S. sarda* is indeed a delicate species that requires ample space and high oxygen saturation rates, it also exhibits a tolerance to higher bioloads that is larger than initially suspected. The fact that three of the five animals packed with two *Dasyatis pastinaca*, one *Torpedo* *marmorata*, one *Pagellus bogaraveo*, one *Seriola rivoliana*, one *Spondyliosoma cantharus*, and three *Octopus vulgaris* survived is proof that these animals are more resistant than originally anticipated. The authors maintain that, however, such scombrid species should preferably be moved with conspecific individuals only, with conditions such as the ones used in this tank to be avoided or limited to investigative purposes only.

Zero mortalities occurred at a bioload of 3.1 kg/m^3 and two out of five mortalities (i.e. 40%) occurred with a bioload of 11.2 kg/m^3. This infers the question whether the two mortalities occurred from excessive bioload or simply from excessive "obstacles" in the water, as scombrids are known for schooling and swimming in a circular pattern while in transit [Farwell, 2001a,b]. Given the fact that, despite the higher bioload of 11.2 kg/m^3 in the tank with five *S. sarda*, water quality showed values similar to those in the tank with four *S. sarda* only (pH being only slightly lesser, i.e. 8.13 vs. 8.28), the authors suggest that higher bioload was not the key factor in the demise of the two animals but, most likely, the presence of excessive other animals, which acted as "obstacles" and prevented a free and unobstructed circular swim pattern. This may have lead to excessive energy expenditure and ultimately death.

After arrival, two other *S. sarda* succumbed and displayed multiple skin abrasions, which resulted in an overall five survivors from the original nine animals packed. Some of the surviving animals also displayed skin abrasions upon arrival, but these were treated by supplementing their diet with the aforementioned large concentrations of multivitamin substances and generic antibiotic ointments applied topically.

These transports also revealed that *S. japonicus* has a high tolerance for relatively higher bioloads, which ranged as high as 18.2 kg/m^3 and still not a single mortality was observed in all 1.869 individuals moved.

High oxygen saturation rates, most likely, also played a key role in preventing mortalities, as the authors endeavoured to maintain values above 200%. Occasional anecdotal rumours circulate amongst the public aquarium industry that excessive high oxygen saturation rates during transport might be detrimental and indeed cause irreversible injury to delicate gill tissues. The authors would like to take this time to state that they have used oxygen saturation rates as high as 400% and have never sustained any mortality in transit, the two *S. sarda* in this article being their first. Previous transports conducted by the same authors with other species, such as *Argyrosomus regius*, *Mola mola*, *M. mobular* [Correia et al., 2008], and *Sphyrna lewini* [Young et al., 2002] have used similar high oxygen saturation rates and still no mortalities were observed. The three transports reported here included multiple teleost, elasmobranch, and invertebrate species (Table 1) and, still, no mortalities were observed despite occasional oxygen saturation peaks as high as 370%.

The use of AmQuel® coupled with pH buffers proved to be a valuable tool in the prevention of ammonia build-up and excessive decrease in pH, as previously reported by the same authors in Correia et al. [2008]. These three trips, however, allowed the conclusion that concentrations around 10 ppm of AmQuel® and anything lower than 30 ppm of pH buffers is clearly insufficient—at least for bioloads in this order of magnitude (i.e. 16.0 kg/m^3). Indeed, the best results were obtained with the third formula (November 1), with 25/40/40 g of AmQuel®/sodium bicarbonate/sodium carbonate, which corresponded to concentrations of 19.2/30.8/30.8 ppm.

Still, the authors speculate that even higher concentrations of pH buffers will assist in maintaining pH truly stable throughout the trip, with virtually no change over time. As such, the authors wish to put forth that a "cocktail" with the following concentrations be subjected to future use and investigation: 20/40/40 ppm, which corresponds roughly to 25/50/50 g in a 1.3 m^3 transport tank.

These findings were further substantiated by Modesto et al. [2009], who conducted parallel studies on the use of AmQuel® and pH buffering agents under a controlled environment. Flying Sharks established a partnership with these researchers, who devised a series of experiments targeted at finding the best "cocktail" formula for live marine animals transport, particularly scombrids. Trials consisted on setting up 2.000 l tanks with ammonia concentrations artificially dosed from 0.25 to 2.50 ppm. A series of different "cocktail" recipes were then tested and the overall best results were obtained with the highest concentrations tested, which were 20/20/10 ppm of AmQuel®/sodium bicarbonate/sodium carbonate.

One interesting point was the sudden drop in pH registered during the August 1 trip at about 5 hr of transport (Fig. 6). These sudden drops are not uncommon, particularly during the early stages of the trip, when animals are still adapting to their captive environment and, therefore, excrete excessive metabolites. On this particular instance, the drop in pH was immediately counteracted by the addition of two, not just one, doses of sodium bicarbonate+sodium carbonate, which elevated pH to its previous value, i.e. around 8.1.

Another significant drop occurred, also during the August 1 trip, from the 12 to 16 hr period, which corresponded to the time filtration that was not operating because the tanks were in flight. After landing, pH was restored by multiple additions of sodium bicarbonate+sodium carbonate. The authors suggest that, in future trips, transport legs where filtration is going to be turned off should be associated to preventive measures, such as the addition of two doses of sodium bicarbonate+sodium carbonate "before" filtration is turned off.

In summary, all results, therefore, strongly suggest that a concentration of 20 ppm of AmQuel® can quench ammonia concentrations as high as 2.50 ppm. Furthermore, both the authors' and Modesto's team results suggest that a single addition of 20 ppm of AmQuel® might very well keep ammonia virtually null throughout the trip, although regular monitoring is advised. pH, however, will consistently drop unless a concentration of at least 40 ppm of sodium bicarbonate and sodium carbonate is added regularly, typically every 3 hr.

These results corresponded to bioloads that ranged, in *S. japonicus*, from 11.7–18.2 kg/m^3 and will most likely need adaptation should higher bioloads be transported.

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