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HUSBANDRY REPORTS

Notes on the Husbandry and Long-Term Transportation of Bull Ray (*Pteromylaeus bovinus*) and Dolphinfish (*Coryphaena hippurus* and *Coryphaena equiselis*)

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Bull rays (*Pteromylaeus bovinus*) and Dolphinfish (*Coryphaena hippurus* and *Coryphaena equiselis*) were collected in Olhão (south of Portugal). These animals hosted multiple parasites, namely *Caligus* spp., and underwent a variety of treatments to remove them. Of all treatments tested, hydrogen peroxide showed the best results, although only concentrations above 100 ppm were effective in parasite removal. These high concentrations, however, proved to be highly toxic for the fish and led to the loss of some animals, especially those which had been handled before treatment. A total of 14 Bull rays were transported to Bolougne-Sur-Mer (France) by road and some animals were lost, which was attributed to excessive time in transit (>45 hr). In another transport, three Bull rays and 10 Dolphinfishes were moved to Stralsund (Germany) by road and air. The mechanical wounds suffered by one of the Bull rays during transport led to its death and, consequently, a deterioration of water quality in the tank containing two other conspecifics. This deterioration of water quality resulted in problems for the other two Bull rays, and one perished approximately 48 hr after arrival. The authors concluded that Dolphinfish can be transported with a low bioload for at least 27 hr, and Bull rays should not undergo transports longer than 35 hr. Special attention must be taken to injured animals, since this can lead to a decrease in water quality and consequently affect other animals in the same transport tank. Zoo Biol. 32:222–229, 2013.

Keywords: collections; water parameters; public aquaria; conservation; parasites

STATEMENT OF THE PROBLEM

In recent years the public aquarium industry has undergone extensive growth, due to advances in aquarium science and increased public interest in the aquatic environment. However, engaging a public that is used to modern technology is no easy task and public aquaria focus increasingly more on acquiring and displaying original and novel animals.

The Bull ray, *Pteromylaeus bovinus* (Geoffroy Saint-Hilaire, 1817), Common dolphinfish, *Coryphaena hippurus* Linnaeus, 1758, and Pompano dolphinfish, *Coryphaena equiselis* Linnaeus, 1758, are but a few of the many examples of animals traditionally considered "difficult" and therefore rank prominently high in the aforementioned list of "original" species.

The Southern coast of Portugal is a passageway for animals swimming in and out of the Mediterranean Sea during their migratory patterns. In 1994 the commercial fishing company Tunipex established a set-net in this location. Flying Sharks, the collections company the main authors are affiliated with, enjoys a close partnership with Tunipex, the result of which being the supply of live marine animals to public aquaria worldwide, as mentioned in previous publications, such as Correia et al. [2008, 2011]. The set-net operating off the Algarve shore captures a diverse array of both teleost and elasmobranch species

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[Correia et al., 2008, 2011], with Dolphinfish and Bull rays being relatively frequent during the warmer summer months.

The majority of these animals host numerous external parasites, namely copepods like the sea lice, *Caligus* spp. These parasites use rasping mouthparts to graze upon the host and remove mucus, skin, and underlying tissues [Costello, 1993]. Impacts on the host's skin include epithelium loss, bleeding, increased mucus discharge, altered mucus biochemistry, tissue necrosis, and consequent loss of physical and microbial protective function [Johnson et al., 2004; Tully and Nolan, 2002]. It is therefore important, perhaps even critical, that animals are cleaned from parasites before traveling, as the trip alone constitutes enough of a stressor without the addition of a parasitic load that undoubtedly hinders an already compromised immune system.

The transportation of live marine fish involves careful planning and logistics, depending on numerous variables as stated previously [Correia, 2001]. Many other authors provide an extensive background on the various aspects involved in these operations [e.g. Correia et al., 2008, 2011; Smith et al., 2004; Young et al., 2002], with ammonia excretion and pH being the traditional main points of concern as, during transport, pH will gradually decrease while ammonia will increase as a result of carbon dioxide buildup and the release of nitrogenous waste and miscellaneous stress-related metabolites, respectively. Both 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—$NaHCO_3$) and/or soda ash (i.e., sodium carbonate—Na_2CO_3). Ammonia (NH_3 and $NH4^+$) 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.

Both *Coryphaena* spp. and *P. bovinus* are sensitive to water quality. Nevertheless, public institutions demonstrate increasing interest in acquiring these species, which created the perfect opportunity to analyze their performance during long-term transports and also deploying the appropriate measures to ensure the trips were successful and all animals arrived in optimal conditions.

DESCRIPTION OF THE PROCESS

Between May and August 2011, numerous animals were collected by Tunipex's and Flying Sharks' staff over multiple days. Among the collected species were *P. bovinus*, *C. hippurus*, and *C. equiselis.* Once caught in the set-net, animals were removed from the ocean using nonabrasive vinyl stretchers, and transported to land by boat inside a 1.6-m diameter round polyethylene vat filled 1.0 m high with seawater, yielding a volume of approximately 1,000 liters. 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 on shore, the fish were immediately—but carefully—moved to a 10.0 m diameter × 1.8 m high round fiberglass tank (Main Tank), again using a vinyl stretcher. This staging tank is on a flow-through system with mechanical and biological filtration units and has no temperature control. Introduction was, therefore, preceded by a quick acclimation period (typically 10–15 min).

TABLE 1. Animal distribution during the parasite removal operation at Flying Sharks' holding station in Olhão

	T1	T2	T3
Coryphaena spp.	7	0	0
Pteromylaeus bovinus	0	6	5

Animals were closely supervised for the rest of the day, in order to assure their adaptation proceeded without any noticeable problems.

Parasite Removal Operations

Sea lice, *Caligus* spp. were visible in almost all specimens, especially *P. bovinus*, and a plan was devised to eliminate them. A total of 11 *P. bovinus* and 7 *Coryphaena* spp. were divided into three 2.4-m diameter round polyethylene tanks (Table 1).

While the treatments were conducted inside the 2.4 m tanks, the main tank—where the fish were originally held—was drained, intensively scrubbed, disinfected with a sodium hypochlorite solution (commercial bleach) and thoroughly flushed with seawater until there were no noticeable remains of sodium hypochlorite.

Pteromylaeus bovinus *treatments*

1. Eleven *P. bovinus* were divided between Tank 2 (six animals) and Tank 3 (five animals). They then went through a 4 hr treatment with 20 ppm Praziquantel (Farmaquimica Sur, Malaga, Spain) and displayed normal behavior during the operation.
2. After the treatment mentioned in (1), the specimens were observed individually and external parasites removed manually with tweezers. Some animals revealed a relatively low bioload of parasites (10–20 individuals) while others revealed a heavy bioload (over 100 individuals). Generally, the larger the animal, the higher the parasite load.
3. After the visual inspection, the animals in Tank 2 were transferred to a 4-m diameter round tank (Tank N) with clean seawater and the animals in Tank 3 were transferred to Tank S, which was similar to Tank N.
4. Since the 4 hr of treatment with Praziquantel apparently had little effect in the removal of parasites, one of

the bigger animals went through a 100-ppm hydrogen peroxide treatment in a 1.6-m diameter round tank and did not undergo the manual parasite removal operation. Immediately after the animal was introduced to this tank the parasites began to leave the host's body surface in a very obvious and visible way—even to the naked eye—and remained in suspension in the water or sit on the bottom of the tank. This treatment lasted for 1 hr. After this, the animal was moved to the Main Tank.

5. The promising results in (4) led the team to subject all animals in Tank S to a 150-ppm hydrogen peroxide treatment. However, unlike the one animal that underwent the 100 ppm treatment described in (4), the animals in the 150 ppm hydrogen peroxide bath revealed immediate abnormal behavior, consisting of unbalanced swimming and very conspicuous tremors of the fins. They were thus promptly moved to the Main tank, with clean seawater and an extra supply of oxygen through a pressurized oxygen cylinder.
6. All animals in Tank N then went through a 100 ppm hydrogen peroxide treatment (lower concentration than in point 5) to avoid similar reaction). However, all rays revealed similar abnormal behavior and were immediately transferred to the Main Tank and assisted by the staff with oxygen supply and swimming.
7. Some minutes after, all rays were swimming normally, with the exception of one animal, which died minutes after its introduction in the Main Tank. This animal, however, had undergone a quick passage through the 150-ppm hydrogen peroxide bath, which ended quickly because it displayed immediate abnormal behavior and tremors.

Three hours later, one of the smaller animals that underwent the 100 ppm hydrogen peroxide treatment also died. The following morning, four more animals were dead.

8. In the same morning, a new ray arrived to the station and went through a 1 hr at 100 ppm hydrogen peroxide bath on a 1.6-m diameter tank. This animal died a few hours later.

Coryphaena *treatments*

1. All *Coryphaena* specimens in Tank 1 went through a 300-ppm formalin bath for 3 hr. The animals were closely monitored and reacted well, hence the long duration of the treatment.
2. Animals were then dipped in a 1.9-m round tank with freshwater (0% salinity) for 5 min. One of the animals started to evidence odd behavior and erratic swimming, quickly lying on the bottom of the tank. These fish were immediately transferred to a container with 100 ppm of the sedative phenoxyethanol (Panreac, Barcelona, Spain) and a visual inspection of their gills was performed, in order to assess their parasite load. Most animals were "clean" of parasites but three revealed the presence of *Penella* sp. These fish were finally moved to a 4-m round tank (Tank E). Of the seven fish that underwent this treatment, five died shortly after.
3. In light of the results described in (2), no more *Coryphaena* spp. were subjected to the freshwater baths nor the visual inspection while immersed in phenoxyethanol. The remaining 14 *Coryphaena* sp. therefore went through a 50 ppm hydrogen peroxide treatment in Tank S (4-m diameter round tank). The animals did not reveal any sign of discomfort, so it was decided to increase the treatment concentration to 62.5 ppm after 45 min. After a 1-hr bath (total duration), all fish were transferred to the Main Tank. During the bath, no free parasites were observed in the water, suggesting that only concentrations of 100 ppm, and above, cause the parasites to actively leave the host's body.
4. The animals referred to in point 2 then went through a 62.5 ppm hydrogen peroxide treatment for 1 hr in Tank N, the same as mentioned in point 3. This treatment also included other fish: one *Pagellus erythrinus*, two *Epinephelus marginatus*, one *Dasyatis violacea*, and one *Lithognathus mormyrus*. None of these fish revealed discomfort with the treatment. The *Coryphaena* were moved back to the Main Tank after the treatment and no losses occurred until transport occurred weeks later.

Animal Transports

A total of 10 *Coryphaena* spp. and 17 *P. bovinus* were transported to two different institutions: 10 *Coryphaena* spp. and three *P. bovinus* went to the Ozeaneum, in Stralsund (northern Germany), and 14 *P. bovinus* went to Nausicaá, in Boulogne-Sur-Mer (northern France). Both transports took place approximately 3 weeks after arrival of all specimens to the temporary holding station in Olhão. Two days before traveling, all animals were fasted to decrease the amount of nitrogenous waste released during transport.

Transport tanks for both missions followed a relatively simple concept previously used and described in detail by Correia [2001], Correia et al. [2008, 2011], and Young et al. [2002]. This simple method (Fig. 1) consists of polyethylene round containers of three different dimensions: 1.75 m diameter × 0.60 m high, 1.9 m diameter × 0.77 m high and 2.4 m diameter × 1.05 m high.

All animals were collected from the temporary holding tanks and moved inside their respective transport tanks with vinyl stretchers (*P. bovinus*) and nets with rubber mesh (*Coryphaena* sp.). Handlers used latex gloves to prevent damage of the skin should any contact occur accidentally. While in transport, animals and equipment were checked approximately every 3 hr. Checks included monitoring the animals, equipment, and

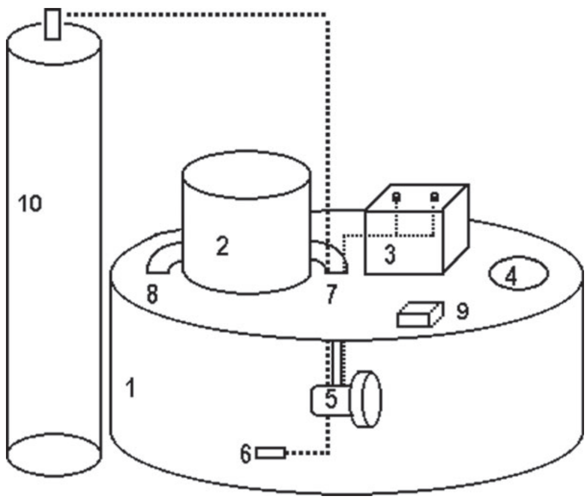

Fig. 1. General design of tank used for marine animals transport by road and air. *Legend*: 1. Polyethylene transport tank (1.75 m diameter × 0.60 m high or 1.9 m diameter × 0.77 m high or 2.4 m diameter × 1.05 m high) with a 15 mm thick fiberglass reinforced wooden lid bolted to a 30 mm gasket. 2. Filter 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. During the *Coryphaena* sp. and *P. bovinus transports*, the access windows were actually substantially larger, measuring 80 × 80 cm in 1.75 m tanks and 120 × 80 both in 1.9 and 2.4 m tanks. The 2.4-m wide tanks also included a foam fractionator (Model AquaC EV-1000, Aquatic Eco-Systems, Apopka, Florida, USA). 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 generally not permitted inside aircrafts, during flight. However, during the *P. bovinus* transport (the only one that included an air leg), oxygen was permitted in flight and this apparatus was therefore not used). 10. Pressurized oxygen cylinder, secured to wooden pallet carrying tank.

water quality parameters, such as temperature, 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 the percent saturation dropped below 150. 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 a cubic meter of water. 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., 20/50/50 g, respectively Amquel, Sodium bicarbonate, and Sodium carbonate) in a 3-L container, shaking it vigorously and dropping the liquid contents directly on the surface of the transport tank water. This proportion of the three reagents yielded different concentrations depending on the volume of each tank, but this formula proved effective in multiple volumes of 1,000 L and above, as described by Correia et al. [2011].

Transport to Ozeaneum (Stralsund, Germany)

The transport to Stralsund lasted 21.5 hr and included a segment by road from Olhão to Lisbon, a segment by air to Leipzig and a third road segment to Stralsund. Additional details are given in Table 2. A total of three *P. bovinus* and 10 *Coryphaena* spp. were moved by road from Olhão to Lisbon on August 27, 2011, then flown to Leipzig and moved by road to Stralsund, with a total trip time of approximately 27 hr. Three 1.75 m diameter × 0.60 m high (Tanks 1, 2, and 3, with individual water volumes of approximately 850 L) and two 2.40 m diameter × 1.5 m high (Tanks 4 and 5, with individual water volumes of approximately 3,200 L) circular tanks were used in this transport. Table 2 includes all animals that were shipped and their tank distribution. However, this article focuses exclusively on the transport of *Coryphaena* and *Pteromylaeu*s. Bioloads were estimated, dividing the total biomass per tank by its respective volume of water.

Transport to Nausicaá (Boulogne-Sur-Mer, France)

The transport to Boulogne-Sur-Mer lasted 40 hr and consisted on a single road trip from Olhão to Nausicaá, with additional details given in Table 3. Fourteen *P. bovinus* were moved in five tanks by road from Olhão to Bolougne-Sur-Mer on August 29, 2011, with a total trip time of 45 hr. Three 1.90 m diameter × 0.77 m high (Tanks A, B, and C, with individual water volumes of approximately 1,000 L and two 2.40 diameter × 1.5 m high (Tanks D and E, with individual water volumes of 3,200 L) circular tanks were used in this transport.

DEMONSTRATION OF EFICACY

Parasite Removal Operations

Pteromylaeus bovinus *treatments*

Visual results on Praziquantel treatments performed on *P. bovinus* suggested it did not seem to have any effect on parasite removal despite the fact that the animals handled the treatment well during its 4-hr duration. On the other hand, the manual process of parasite removal seemed very effective and allowed the staff to remove close to 100% of all visible parasites.

TABLE 2. Animal distribution per tank on road and air shipment done from Olhão (Portugal) to Stralsund (Germany) on 27–28 August 2011

	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5
Tank diameter (m) >	1.75	1.75	1.75	2.40	2.40
Bioload (Kg/m^3) > Species	27.02	16.02	2.72	10.94	1.88
Apogon imberbis			10		
Chelon labrosus			20		
Coris julis			2		
Coryphaena spp.					10
Mullus surmuletus			20		
Octopus vulgaris		3			
Pagrus pagrus			2		
Pteromylaeus bovinus		1		2	
Sarpa salpa			46		
Scomber japonicus	215				
Seriola rivoliana		4			
Thalassoma pavo			16		
Trachinotus ovatus			1		
Trachurus trachurus	16				

The hydrogen peroxide treatment seemed effective for sea lice. However, it was very aggressive to the animals, even in small concentrations (100 ppm), leading to seven losses out of a total of 12 animals.

Coryphaena *treatments*

All Dolphinfish went through a 300-ppm formalin bath that, apparently, had no effect on the parasites. The same happened with the animals that went through a freshwater bath. However, unlike the formalin bath, these animals did not react well to freshwater and were removed shortly after being introduced due to the observed abnormal behavior. This operation led to the loss of five animals out of seven.

The hydrogen peroxide treatment also showed no significant effect on parasite removal but did not cause any losses.

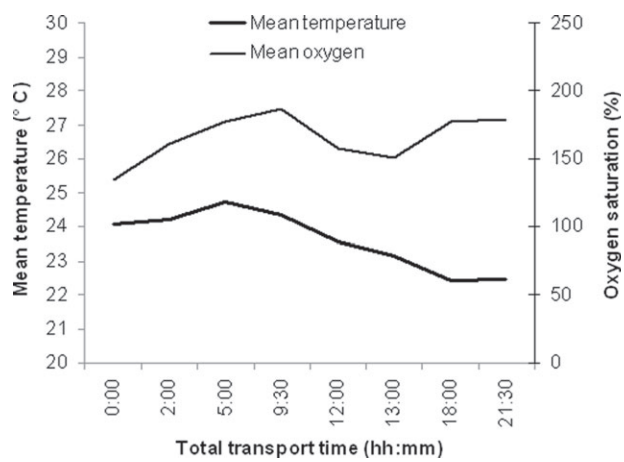

Fig. 2. Temperature and oxygen saturation during road and air transport from Olhão (Portugal) to Stralsund (Germany) in August 2011. Values shown are the mean of parameters from all five tanks. Standard deviations for temperatures were between 0.24 and 0.80 and for oxygen were between 43.22 and 102.60 during multiple readings.

Animal Transport

Transport to Ozeaneum (Stralsund, Germany)

All animals arrived at the Ozeaneum in good health, except for one Bull ray. This animal was in Tank 2 (Table 2) along with the three *Octopus vulgaris* (each of these three animals were kept in separated cages) and four *Seriola rivoliana*. The Bull ray started to evidence stress and mechanical abrasions on the rostrum about 8 hr before reaching its final destination. As such, the team decided to move it to Tank 4, with two other conspecifics. It perished, however, approximately 4 hr after arrival. Another Bull ray died 48 hr after arrival. Water quality parameters of the transport are given in Figs. 2 and 3. The addition of oxygen kept its saturation rate above 100% at all times (Fig. 2). The addition of AmQuel and sodium bicarbonate successfully

TABLE 3. Animal distribution of animals per tank on road shipment done from Olhão (Portugal) to Boulogne-Sur-Mer (France) on 29–30 August 2011

	Tank A	Tank B	Tank C	Tank D	Tank E
Tank diameter (m) >	1.90	1.90	1.90	2.40	2.40
Bioload (Kg/m^3) > Species	19.53	18.21	16.70	21.56	22.50
Pteromylaeus bovinus	2	2	2	4	4

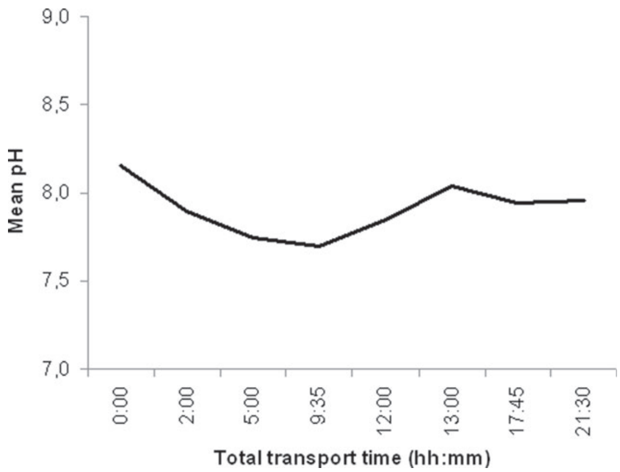

Fig. 3. pH during road and air transport from Olhão (Portugal) to Stralsund (Germany) in August 2011. Values shown are the mean of parameters from all five tanks. Standard deviations were between 0.19 and 0.50 during multiple readings.

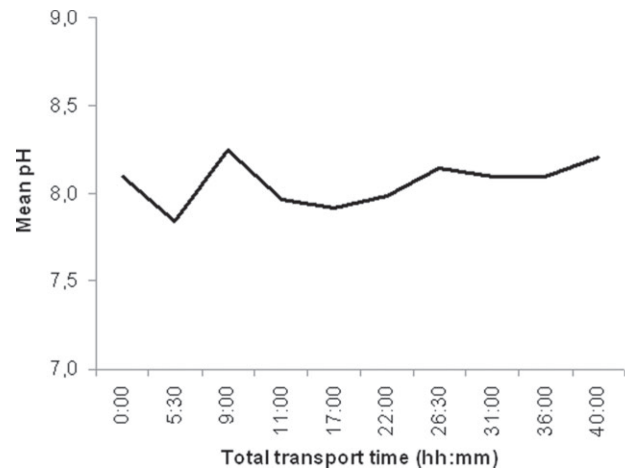

Fig. 5. pH during road and air transport from Olhão (Portugal) to Boulogne-Sur-Mer (France) in August 2011. Values shown are the mean of parameters from all five tanks. Standard deviations were between 0.00 and 0.22 during multiple readings.

kept pH above 8.0. (Fig. 3) and ammonia under 0.25 ppm. All surviving animals, at this point, remained so for at least multiple months.

Transport to Nausicaá (Boulogne-Sur-Mer, France)

Of all 14 animals transported one perished in transit (at approximately 36 hr of total transit time) and was removed from its tank (D). Once the destination was reached the animals were transferred from their transport tanks to Nausicaá's facilities. Abnormal behavior was noticed in three other animals with evidence of stress and erratic swimming. These three animals along with two others perished within 48 hr after arrival, despite the fact that antibiotics were administered intramuscularly to all 13 live animals within 8 hr after arrival (Enrofloxacyn at 15 mg/Kg). The addition of oxygen kept its saturation rate above 100% at all times (Fig. 4). The addition of AmQuel and sodium bicarbonate successfully kept pH at approximately 8.00 (Fig. 5) and ammonia under 0.00 ppm. All surviving animals, at this point, remained so for at least multiple months.

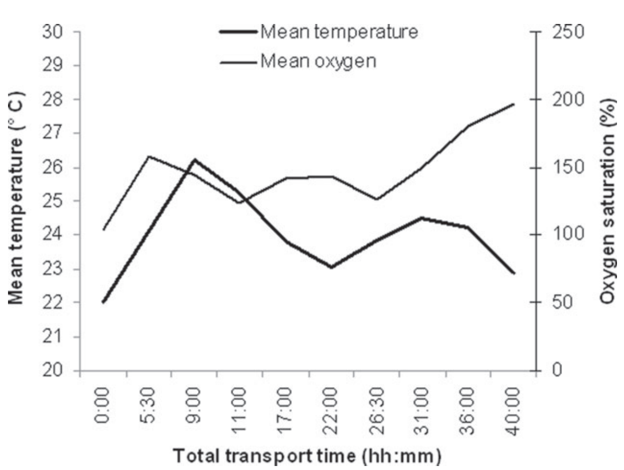

Fig. 4. Temperature and oxygen saturation during road and air transport from Olhão (Portugal) to Boulogne-Sur-Mer (France) in August 2011. Values shown are the mean of parameters from all five tanks. Standard deviations for temperatures were between 0.00 and 0.66 and for oxygen were between 0.00 and 88.89 during multiple readings.

Regarding treatments on *Pteromylaeus bovinus*, the Praziquantel bath (4 hr at 20 ppm) revealed to be ineffective in the removal of *Caligus* spp. despite the fact that the Bull rays showed no signs of discomfort during the treatment. The authors discovered later that Praziquantel is very effective in the elimination of plathyelminthes, but not of copepods [Noga, 2000], and regret not having investigated this matter before subjecting the animals to this treatment. The use of hydrogen peroxide, on the other hand, seemed to be efficient based on the positive result evidenced by the one animal that did not undergo any previous treatment, nor handling, and went through a 60 min treatment at 100 ppm. However, all animals that had been handled before an hydrogen peroxide treatment (both at 100 and 150 ppm) showed signs of stress followed by death in seven of 12 cases. In light of these observations, the authors recommend that hydrogen peroxide is not used in elasmobranchs in conjunction with any other treatment, as excessive handling seems to render the animals unfit to endure hydrogen peroxide baths. These, when conducted on their own, yielded very positive results, although the sample size for this conclusion is limited to one single animal. As such, it might be better advised to avoid hydrogen peroxide treatments on this species and remove parasites manually, which proved to be very effective.

As for *Coryphaena* spp. treatments, formalin did not remove any parasites and the freshwater treatment yielded a very quick response of strong discomfort for the animals. Further investigations revealed that *Caligus* spp. can, in fact, survive in freshwater up to 1 week [Noga, 2000]. The hydrogen peroxide treatment for 1 hr at 50–65 ppm was inconclusive since no exam was performed on the gills after the treatment. One may speculate, however, and based solely on the positive results of the ray that went through the 60 min at 100 ppm, that it may have eliminated some parasites. As for *Coryphaena*, we recommend that, in the future, 100 ppm treatments can be tried again, but never after handling the animals.

Regarding the transports, the method used for both Dolphinfish and Bull rays traveling to Stralsund had been used by the authors multiple times before [Correia et al., 2008, 2011], often using the same exact routing to the same destination, and the loss of one *P. bovinus* was quite unexpected. This was attributed, though, to limited space and excessive "stimulus" during the trip. In retrospect, the authors regret having transported the animal in a 1.75-m wide transport tank—rather than in a 1.9-m wide tank—and the fact that the three live Octopus and four *Seriola rivoliana* were packed together. While in transport Bull rays generally sit on the bottom and remain motionless throughout the trip, but the presence of these other animals most likely prevented the ray from resting and drove it to move constantly in the tank. This is highly undesirable for an elasmobranch that's often sessile in such conditions, and most likely drove it to expend excessive energy and ultimately to its demise.

The moving of this already compromised animal, before dying, to the larger 2.4 m tank already containing two animals seemed appropriate, at first, since it would eliminate the source of discomfort in the previous tank (i.e., the Octopus and Amberjacks) and also expand the area of its confinement from 1.75 to 2.4 m diameter. However, the animal was too compromised at the time it was moved (at approximately 20 hr total trip time) and its death in the new tank further compromised one of the two animals previously traveling inside it.

All Dolphinfish traveling to Stralsund handled transport well and no losses were recorded during, or after, the trip. This method had been used by the same authors in 2010, while moving two rather large (approximately 0.8 m total length) *Coryphaena hippurus* to the Ozeaneum in Stralsund also.

The transport of Bull rays to Boulogne-Sur-Mer had very high water quality throughout the trip mostly thanks to multiple additions of the Amquel/Bicarbonate/Carbonate "cocktail" throughout. This ensured that pH was never below 8.0 and ammonia never above 0.00 ppm. Also, oxygen was never below 100% and temperature was always between 22 and 25°C. The animals were also packed with a relatively low biolad (approximately 18 Kg/m^3 in the 1.75 m wide tanks and approximately 21 Kg/m^3 in the 2.4-m wide tanks), which ensured that the filtration units provided sufficient filtration to ensure that water clarity, among the aforementioned parameters, was also pristine. Still, the animals began to show signs of discomfort within 36–40 hr of total trip time. The loss of one animal in transit, coupled with the further losses of five more within 48 hr after arrival, drove the authors to strongly suggest that trip times be maintained under 36 hr, as these animals seem unfit to endure longer trips in a confined space, despite the fact that water quality was at its best.

This latter suggestion was confirmed in late October (2011), when a truck packed with 10 Bull rays left Olhão (Portugal) to Istanbul (Turkey). This transport was commissioned by the client to a third party who provided adequate mechanical filtration (also based on activated carbon) on two 3,500 L systems, packed with five Bull rays each (yielding a bioload of approximately 15 Kg/m^3). The transporter, however, disclosed that the Bull rays showed great discomfort after 40 hr on the road. This lot of animals subsequently died during a stopover in the Netherlands before proceeding to Turkey.

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