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Capture, husbandry and long-term transport of pilotfish, *Naucrates ductor* (Linnaeus, 1758), by sea, land and air

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Abstract This is a report on the capture, transport, and husbandry of pilotfish, *Naucrates ductor*. The objective of this work was to evaluate the most adequate process for capturing, transporting and maintaining pilotfish, while gaining understanding on their behavior. Collection was done in the Azores, by hook and line. Seventy six individuals were then transported to shore, where they were maintained for two months. After this, they were transported by sea over four days. This transport was then followed by an eight day transport by road along Portugal, Spain and multiple public aquaria in France. The animals endured this trip with no losses and multiple notes on husbandry and behavior are provided. Subsequently (2014 to 16), other animals were shipped by air to Atlanta (Georgia, USA), Plymouth (UK), Budapest (Hungary), Springfield (Missouri, USA), and Dubai (UAE), which involved multiple trials prior to the first shipment, to ensure survivorship and wellbeing during the long transit times. The trials revealed that shipping must occur in the presence of an ammonia quencher and pH buffering agents, including the addition of povidone-iodine to decrease bacterial growth, and ice, to keep temperature low.

Keywords Husbandry · Public aquarium · Conservation · Education · Pilot-fish · Pilot fish

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Introduction

One of the main objectives of displaying marine live animals in public aquaria is to assist these institutions in their mission, which is to inspire conservation and education, as well as displaying the phenomenal diversity of marine species, while recreating their closest possible natural habitat. However, engaging the public - especially in modern days and inherent abundance of technological stimuli - is no easy task, and public aquaria focus increasingly more on acquiring and displaying original and novel animals. On that note, since the introduction of elasmobranchs to public aquaria, pilotfish have been the subject of a keen interest amongst the public aquaria staff although very few have succeeded in introducing it to a captive environment. The distinctiveness and utter visual presence of this species swimming along sharks, as it occurs frequently in nature, renders it a very interesting candidate for public display and therefore merited the authors' attention.

The pilotfish, *Naucrates ductor* (Linnaeus 1758) is an epipelagic oceanic Carangidae species with a circumtropical distribution. It occurs in the Atlantic Ocean, including the Mediterranean, the Pacific and throughout the Indian Ocean (Smith-Vaniz 1986). Typical coloration of pilotfish includes approximately eight vertical bars (Fig. 1), including those on the head and caudal fin, on a light silvery body (Magnuson and Gooding 1971). The characteristic black vertical bars fade away with age, eventually disappearing, leaving the fish with a silvery-white appearance.

The biology and ecology of this species is poorly known, although it is usually one of the most charismatic pelagic species within assemblages associated with fish aggregating devices (FADs) (Relini and Relini 1994; Reñones et al. 1998, 1999). While juveniles are traditionally associated with floating and anchored objects, jellyfish and drifting seaweed, adult pilotfish have a commensal relationship with large-sized living animals, such as sharks, mobulid rays, bony fish and turtles (Massuti and Reñones 1994; Vassilopoulou et al. 2004). This relationship includes swimming along the trail left by, or in front and along of, the sides of larger fish (Magnuson and Gooding 1971), thus reducing energy expenditure (Fish 2010). They mostly feed on scraps of the host's leftovers, parasites and excrements, but also on small fish and invertebrates. Maturation occurs during their first year of age (Vassilopoulou et al. 2004) and the maximum length given for this species is 60 cm FL (Smith-Vaniz 1986). Although maximum age is unknown, it is most likely greater than four years (Reñones et al. 1999; Vassilopoulou et al. 2004). The only available data on the life span of *Naucrates ductor* is based on a few individuals collected by dolphinfish (*Coryphaena hippurus*) commercial fishermen operating in the western Mediterranean, which concluded these animals reached a maximum age of three years (Reñones et al. 1999).

Fig. 1 Pilotfish, *Naucrates ductor*, photographed at the Porto Pim Aquarium in Faial, Azores

The collection of the pilotfish referred to in this study took off Horta, Azores (Fig. 2). The Azores is a Portuguese archipelago composed of nine islands, with an EEZ (Economic Exclusive Zone) encompassing nearly one million square kilometers and marine resources playing a central role in the local economy. With the absence of a continental shelf and surrounding great depths, fishing occurs around the island slopes and multiple seamounts present in the area.

While in transport, multiple issues need to be addressed in order to ensure water quality and animal welfare. One of the main concerns is the decrease in dissolved oxygen in the water as a direct result of respiration. When transport occurs by road, this is easily counteracted by supplying oxygen through the use of an air-stone connected to a cylinder of compressed medical-grade oxygen (Correia et al. 2008). When transport occurs by air, the water needs to be supersaturated with oxygen to a saturation rate that may go as high as 300%, before at least half of the volume of the transport bag – above the water in which the animal is moved - is filled with pure oxygen, before the bag is sealed for transport.

Other concerns include the increase in ammonia and gradual pH decline due to the release of nitrogenous waste and miscellaneous stress-related metabolites and carbon dioxide buildup, respectively (*op. cit.*). Such tendencies may be counteracted through the use of filtration systems or chemical supplements, such as sodium carbonate and sodium bicarbonate to help increase pH, or AmQuel™ (Novalek, Inc., Hayward, CA) to decrease ammonia levels, although slightly lowering pH also. The chemical background behind the use of these substances was discussed at length by some of the co-authors in Correia et al. (*op. cit.*), Correia et al. (2011), and Rodrigues et al. (2013).

This species proved to be a challenge due to the fact that it was the first time it underwent such long transports (both by road and air) and there was a lack of information about their biology and captive behavior. These pilotfish also proved to be extremely sensitive to multiple factors and great care was required while maintaining them in captivity.

The objective of this work was therefore to investigate and conduct the collection, maintenance, transport (by sea, road and air), introduction and acclimation of pilotfish to public aquaria, while addressing the issues highlighted before.

Materials and methods

2013: Sea and road transport

Collection and acclimation

The first lot of 80 animals was collected over the month of August of 2013 along the Condor's Bank (Fig. 2). Blue sharks, *Prionace glauca*, were lured near the boat using a bigeye tuna head (*Thunnus obesus*) as bait, and *Naucrates ductor* were fished with regular fishing line with a hook and bait. Barbless hooks were used, to minimize damage, and an assortment of squid, shrimp and mackerel was used as bait. Once collected, all animals were placed in a plastic container measuring 110 cm in length, 50 cm in width and 35 cm deep in seawater. All individuals were then transported to the Porto Pim Aquarium, in the island of Faial, over a four hours long trip, during which the water in the container was flushed continuously with oceanic seawater.

Once at the Porto Pim Aquarium, all fish were acclimated for 15–20 min to 200 cm diameter fiberglass tanks, while swimming inside 40 × 60 cm plastic bags used to transfer them from the transport container to the quarantine tank. They then remained in quarantine for approximately three months. Each tank was equipped with its own set of filtration, allowing for a fully closed circuit if necessary, consisting of a sand filter, ultra-violet lamps and a biological filter. The fish were fed with an unspecified assortment of shrimp, squid, mackerel and mussels during their quarantine period, and began to feed one day after arrival. These individuals displayed voracious appetite and were fed two times per day to satiation. Approximately 100% of the water volume was changed daily and, for that reason, ammonia concentration wasn't recorded. Oxygen, temperature and pH were recorded two times per day.

Whenever external wounds were visible, the animals were placed in a bath containing 0.1 mg/L of povidone-iodine 10% (1% available iodine, commercial name Betadine™), which proved extremely effective in the healing of abrasions induced by capture and also in the prevention of such lesions during transport. These baths lasted for 48 h, after which the animals rested in untreated water for 24 h. A second 24 h bath of Betadine™, using the same concentration, followed the 24 h rest period. This course of treatment was done once per week, and was repeated two or three times until all external lesions were absent.

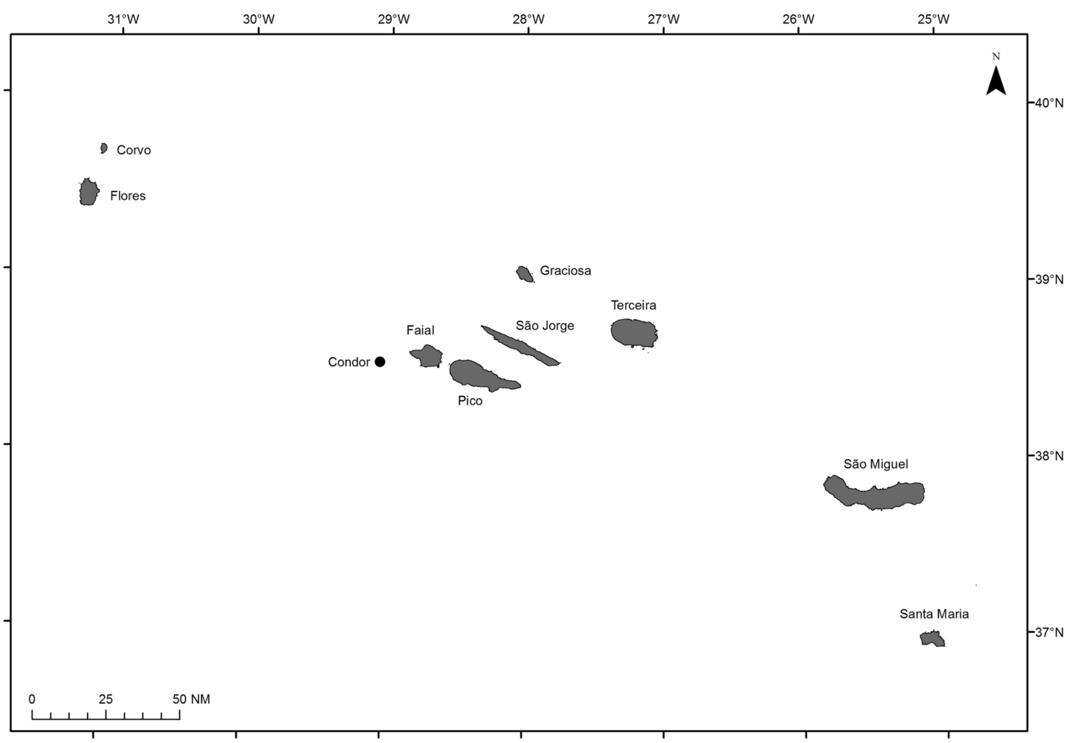

Fig. 2 The Azores Archipelago. Circle southwest of Horta shows location of *Naucrates ductor* collections

Table 1 Number of *Naucrates ductor* - and other specimens from other species - shipped during the long-term transport by road, throughout several public aquaria in Europe in November 2013. 60 individuals were distributed amongst five institutions, 16 remaining through the end of the trip until the Peniche holding station, due to a cancellation while in transit

Date	Number of specimens/species	Location
21 Nov 2013	see list below	Horta Total road distance traveled 0 km
25 Nov 2013	see list below	Leixões Total sea distance traveled 1250 nm Total road distance traveled 0 km
28 Nov 2013	***Naucrates ductor* 20** *Dasyatis pastinaca* 2 *Holothuria forskali* 100 *Mullus surmuletus* 10 *Paracentrotus lividus* 200 *Polyprion americanus* 3 *Thalassoma pavo* 10 *Trachinotus picturatus* 20	Cinèaqua/Paris Total road distance traveled 2460 km
29/30 Nov 2013	*Dipturus batis* 2 *Mullus surmulteus* 4 *Pagellus bogaraveo* 6 *Scorpaena maderensis* 2 *Sparisoma cretence* 2 *Trachurus picturatus* 20	Nausicaá/Boulogne-Sur-Mer Total road distance traveled 2714 km
29/30 Nov 2013	***Naucrates ductor* 5** *Abudefduf luridus* 10 *Anthias anthias* 15 *Apogon imberbis* 30 *Coris julis* 40 *Polyprion americanus* 1 *Scorpaena maderensis* 10 *Sparisoma cretense* 10 *Thalassoma pavo* 40 *Trachurus picturatus* 40	Zee Aquarium Total road distance traveled 2714 km + 449 km (Boulogne to Bergen aan Zee)
29/30 Nov 2013	***Naucrates ductor* 12** *Polyprion americanus* 1 *Zeus faber* 2	Sea Life Weymouth Total road distance traveled 2714 km + 793 km (Boulogne to Weymouth)
29/30 Nov 2013	***Naucrates ductor* 6** *Mullus surmuletus 4* *Ophidiaster ophidianus 3* *Paramolacuvieri 3* *Scyllarides latus 1* *Serranus artricauda 2* *Sparisoma cretense 2* *Sphaerechinus granularis 15* *Thalassoma pavo 6*	Sea Life Oberhausen Total road distance traveled (Boulogne to Oberhausen)

Table 1 (continued)

Date	Number of specimens/species	Location
30 Nov 2013	***Naucrates ductor* 7**	Oceanopolis/Brest Total road distance traveled 3393 km
30 Nov 2013	*Polyprion americanus 3* *Paramola cuvieri 2*	Le Croisic Total road distance traveled 3663 km
30 Nov 2013	***Naucrates ductor* 10** *Zeus Faber 2*	La Rochelle Total road distance traveled 3834 km
30 Nov 2013	***Naucrates ductor* 16**	Peniche holding station Total road distance traveled 5213 km

Transport

On the 20th of November (2013), a group of 76 pilotfish, as well as other teleost and elasmobranch specimens (Table 1), were transported from the Azores to northern mainland Portugal (Leixões) by sea, over a four day journey inside a 12.2 m shipping container, more regularly known as a '40 foot container'. They were then transported by road to multiple public aquaria in France (Table 1). Transport in the shipping container was done in two 190 cm and one 240 cm round polyethylene tanks (Table 2), with a fiber-glass reinforced 15 mm thick wooden lid that was bolted to the rim, ensuring it was leak proof, and 60 × 80 cm & 80 × 120 cm acrylic hatches, respectively, that allowed access to the tanks. The 190 cm tanks had an operational volume of approximately 1.700 L and carried 25 (small) and 11 (larger) pilotfish each, but no specimens from other species. The 240 cm tank had an operational volume of approximately 3.000 L and carried 40 medium sized pilotfish, also without other specimens from other species. Small animals measured approximately 10–15 cm TL; medium sized animals 15–20 cm TL; large animals measured 20+ cm TL. Once arrived to Leixões, on the 25th of November, the container was offloaded from the ship and loaded onto a truck that travelled through Portugal, Spain, and France, distributing all animals as per described in Table 1 and illustrated in Fig. 3.

While on the road, one institution (not listed) cancelled its previous request for animals and 16 pilotfish therefore returned to Portugal (Peniche) on the 2nd of December. These animals were then kept for approximately two months, before being shipped to the Oceanário de Lisboa by road over a one hour long transport. During their week-long road-trip through Spain and France, all tanks were siphoned daily, using a clear hose, once per day, and water quality parameters were measured and recorded approximately every three hours. Temperature and dissolved oxygen were measured using a hand held

Table 2 Water quality results while transporting *Naucrates ductor* by sea and road in November 2013

Location	Tank (diameter)	# Naucrates ductor	O_2 (% sat.)		pH		Temperature (°C)	
			Min.	Max.	Min.	Max.	Min.	Max.
Porto Pim Aquarium	3 tanks of 200 cm	20 to 25 (each tank)	73	101	7,68	8,03	18,2	22,3
Sea and road transport	190 cm	25	78	366	7,15	8,14	13,4	19,9
Sea and road transport	190 cm	11	68	418	7,26	8,07	13,3	20,1
Sea and road transport	240 cm	40	67	375	6,90	7,97	13,4	20,0
Peniche holding station	240 cm	8	90	101	7,60	7,92	14,6	19,1
Peniche holding station	240 cm	8	93	102	7,70	8,00	14,1	18,7

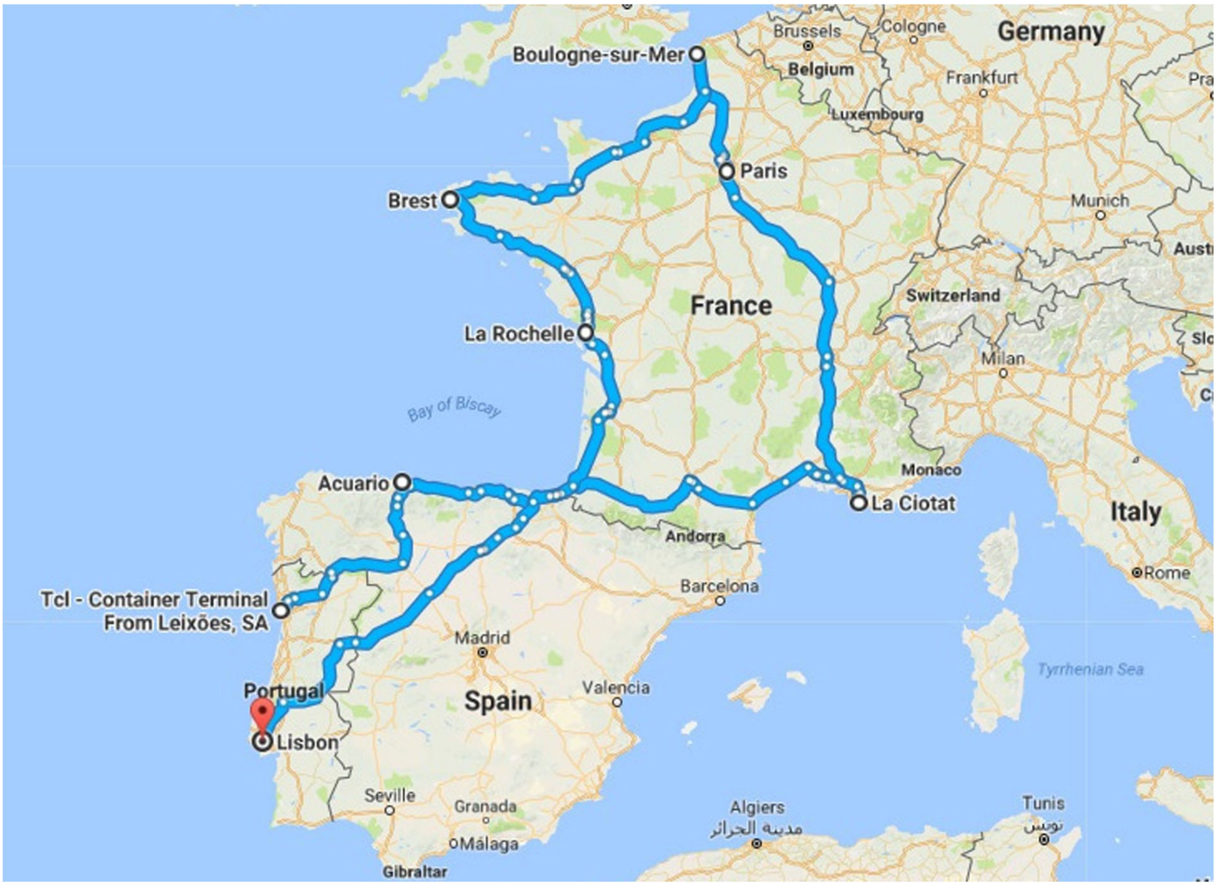

Fig. 3 Route taken when delivering *Naucrates ductor* by road in November 2013. A – Lisbon; H - Leixões; C – La Ciotat (Marseille); D – Paris; E – Boulogne-Sur-Mer; F – Brest; G – Le Croisic; I – Peniche. Since these countries are all members of the European Union, the only documentation necessary for this transport was a TRACES (TRAde Control and Expert System) certificate, issued by the Portuguese authorities

OxyGuard Handy Oxygen probe (OxyGuard Intl., Denmark), pH was measured using a hand held OxyGuard Handy pH probe (OxyGuard Intl., Denmark), and ammonia was measured using colorimetric test kits (Tropical Marine Centre, UK).

Oxygen was typically supplied at a rate of 1–2 L/min and this flow was raised if the saturation rate dropped under 150%. Ammonia (total ammonia nitrogen) test results higher than 0.25 mg/L were immediately counteracted by adding a dose of 25 g of AmQuel™ to the water. Each dosing of AmQuel™ was followed by a dose of 50 g of sodium bicarbonate and 50 g of sodium carbonate, since the use of AmQuel™ is known to be associated with a decrease in pH. This 'cocktail' of 25 g of AmQuel +50 g of sodium bicarbonate +50 g of sodium carbonate was calculated with the objective of quenching 1 mg/L of ammonia in approximately 1.000 L of water and was first introduced by Correia et al. (2008).

Each transport tank was equipped with a mechanical filter that contained a cartridge coated with pleated 50 μm laminated paper sheets and approximately 1.0 kg of activated carbon. Water was driven through each filter, and returned to its tank, by a 220 Volt 8.000 L/h submersible pump, which were in turn powered by a gasoline operated generator fitted with an exhaust pipe that led to the outside of the shipping container, to avoid buildup of toxic combustion fumes inside. Airlines fitted with air-stones provided air continuously to each tank, through an air-pump that was also powered by the generator.

Despite the fact that feeding animals during transport is generally not done, to avoid water quality deterioration, pilotfish could not go without food for 13 days, so food was offered during each stop at each aquarium, allowing for siphoning and a 50% water change a few hours after feeding. Each feed consisted of 400 g of chopped frozen shrimp, squid, mussels and smelt (*Atherina boyeri*), which were thrown to the water surface, where the pilotfish would eat until satiation. Out-flowing water, during each water change, was directed to each institution's reclaimed water system, therefore ensuring the out-flowing water was properly disinfected as per each institution's sewage treating facility.

The introduction of the pilotfish at each institution consisted of carefully acclimating their water to the receiving institutions' water parameters, and then moving them inside plastic bags containing water, with hands continuously covered by latex gloves. Animals were introduced to quarantine tanks and not to exhibits.

Maintenance in Peniche

Once arrived to Peniche, the remaining 16 undelivered *Naucrates ductor* were kept in two 240 cm wide tanks, similar to the largest tank where they were transported in. Filtration of these temporary holding tanks also consisted of a cartridge filter equipped with 0.5 kg of activated carbon that was changed once per week, but it also included a protein skimmer mounted on each lid, that was cleaned every five days. Water quality was monitored and recorded twice daily (morning and afternoon) using the same equipment that was used during the road-trip, except for ammonia parameters, which was not recorded due to the partial 70% water changes performed daily, using saltwater from the surrounding commercial port of Peniche. Out-flowing water from the water changes was directly discharged in the port's sewage system.

The fish were fed twice daily, each feed consisting of the same 400 g of chopped frozen shrimp, squid, mussels and smelt that were used in transport and even prior, at the Porto Pim Aquarium.

During their stay in Peniche no pH buffering agents 'cocktails' were used due to stable pH and ammonia values. Oxygen saturation was kept at approximately 100% through air-stones powered by an air-pump, so no oxygen was added as well. During this time, three fish were lost on the 25th of December, due to an accidental power failure and consequent water deterioration. On the 30th of January (2014), the remaining 13 pilotfish were transported to the Oceanário de Lisboa, a large public aquarium. As before, all fish were captured using plastic bags after lowering the water level to approximately 30 cm deep. They were then transported in two 160 cm (diameter) polyethylene round tanks, similar to the ones used in transport and with similar lids and filtration units, which were not used given the shortness of the trip. The water used was provided by the tanks where the fish had been staying, and only oxygenation was provided to the transport tanks. All fish arrived well after a one hour long road trip.

2014–2016: Air transports

During the summers of 2014, 2015 and 2016 additional lots of pilotfish were collected in the same exact location as the ones collected in 2013, using similar methods. Transport to shore was done inside a round 1.35 m diameter polyethylene tank and water was flushed continuously throughout the four-hour long trip to shore. Acclimation and holding at the Porto Pim Aquarium consisted of identical steps as the ones highlighted before, for 2013. Unlike the animals collected and transported in 2013, the lots from 2014 to 2016 were shipped to multiple destinations by air inside sealed styrofoam boxes, in accordance with IATA (International Air Transport Association) Live Animals Rule 51 (Fig. 4). A series of trials was therefore previously devised to assess the correct method through which these animals could travel inside sealed plastic bags, one animal per, measuring 80×120 cm each, from the Azores to Lisbon, and finally to their end destination.

The trials involved multiple volumes of water, multiple lengths of time inside the sealed bag, multiple oxygen saturation rates and also the addition – or not – of Amquel™ and pH buffering agents. Some of the trials included the addition of the aforementioned povidone-iodine 10% (1% available iodine, commercial name Betadine™) treatment, as it proved extremely effective in the healing of post-capture external abrasions and also in the prevention of such abrasions during transport. A standard 'off the counter' solution of Betadine™ was applied in the water at the concentration of 1 ml per 100 L. Details of all trials are provided in Table 3.

During these animals' stay at the Porto Pim Aquarium, they were subjected to a Betadine™ bath similar to the one described for the 2013 initial lot of animals, although with a different time-table: the initial bath was 48 h long, to which a 24 h resting period in 'clean water'

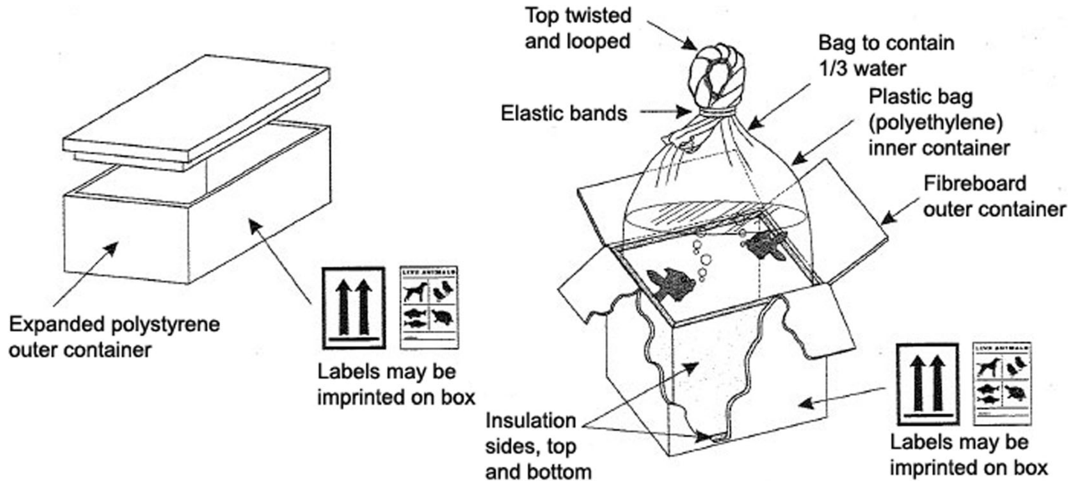

Fig. 4 IATA (International Air Transportation Association) Live Animal Regulation number 51, for transporting live fish inside plastic bags, that travel inside styrofoam boxes that, in turn, must travel inside a cardboard box

followed, completed with a second 48 h bath in Betadine™.

After initial discussions with a freight-forwarding agency, it became apparent that the total transit time from Horta to Atlanta (USA), the first destination, would have to include an overnight layover in Lisbon and then a second overnight layover in Frankfurt, therefore adding to more than 60 h of total transit time, potentially more if any delays were encountered. Based on the results obtained with the trials described above, this was deemed as an excessive transit time, so a decision was then made to stage the animals at the Superior School of Sea Technology in Peniche (one hour north of Lisbon), after their arrival from the Azores. Once the animals were properly acclimated from their initial 12–16 h trip – inbound from the Azores - they would then be transported by road to Lisbon airport, from which they would fly to Atlanta via Frankfurt.

Since this route proved to be too long, after a first shipment of five fish on the 17th of December 2014, a second route via Madrid was devised, whereby seven animals were transported by road to Madrid, on the 3rd of February 2015, using the exact same method previously described for road transports, albeit inside a smaller (140 cm wide) polyethylene tank, equipped with the same mechanical filtration. The animals were then packed in situ (i.e. at Madrid airport). Once bagged and boxed, they were flown on a direct flight to Atlanta, therefore cutting the total trip time, inside the plastic bags, to 22 h. Details on these transports are also provided in Table 3. In both transports the fish were bagged inside the aforementioned 80 × 120 cm plastic bags, containing 40 L of water each, and then packed inside styrofoam boxes measuring 78 cm long × 57 cm wide × 40 cm tall. Each plastic bag with water, oxygen and fish was double bagged inside a second plastic bag, which was then wrapped in a third black opaque rigid half-bag, covering only the water portion, which allowed for further mechanical and also visual protection, enabling the animals to remain calmer.

This packing method was used throughout all subsequent shipments and the route via Madrid was used once again on the 28th of October 2015, when 20 animals were shipped to Springfield (Missouri, USA), via a direct flight from Madrid to Chicago, immediately after arrival from Horta by air, which yielded a total transit time of 34 h, that included an 11 h drive from Chicago airport to the end destination in Springfield.

On the 5th of October 2015, a lot of 35 individuals were moved from Horta to Lisbon by sea, over five days, using identical methods to the ones described above for 2013, although the animals were substantially smaller, with weights between 100 g and 200 g. Once the ship docked in Lisbon, 20 pilotfish were packed (one per bag, two per box) inside boxes similar to the ones used before (78 cm long × 57 cm wide × 40 cm tall), after wish they were delivered to the airport, from which they then flew to London (approximately 3 h) and were then driven to Plymouth (2 h). The day after, 15 fish were packed in Peniche, after an overnight stay at the

Table 3 Water quality results for simulated and effective transports of *Naucrates* ductor by air during 2014, 2015 and 2016. Transports to European Union countries were accompanied by a TRACES certificate, issued by the Portuguese authorities. The transport to the UAE was accompanied by a Certificate of Origin, Veterinarian Certificate, and CITES exemption declaration, all issued by the appropriate Portuguese authorities. Transports to the USA were accompanied by a CITES exemption declaration, this being the only requirement by the USA Government. '- - -'denotes information not recorded

Date	Origin - route - destination	Time in transit (hr)	# *N. ductor*	Biomass (g)		Bioload (kg/m^3)		D.O.A.	D.O.A. after 24 h	Water volume (L)	Fasting (hr)
				Min.	Max.	Min.	Max.				
1 Oct 2014	Trial transport (in Horta)	13	2	400		16,0		0	0	25	48
15 Oct 2014	Trial transport (in Horta)	24	2	400		13,3		0	0	30	96
22 Oct 2014	Trial transport (in Horta)	37	4	400		11,4		0	0	35	96
10 Nov 2014	Trial transport (in Horta)	36	2	400		10,0		0	0	40	96
12 Dec 2014	Horta – Lisbon – Peniche	16	5	90	430	2,4	11,6	0	0	37	72
17 Dec 2014	Peniche – Lisbon – Frankfurt – Atlanta	42	5	90	430	2,4	11,6	3	0	37	72
28 Jan 2015	Horta – Lisbon – Peniche	12	7	300	500	7,5	12,5	0	0	40	72
3 Feb 2015	Peniche – Madrid – Atlanta	22	7	300	500	7,5	12,5	1	+2	40	72
5 Oct 2015	Horta – Lisbon – London – Plymouth	24	20	100	200	5,6	11,1	0	0	18	72
6 Oct 2015	Horta – Lisbon – Budapest	18	16	100	200	5,6	11,1	0	0	18	72
28 Oct 2015	Horta – Lisbon – Madrid – Chicago – Springfield	34	20	100	200	5,6	11,1	0	0	18	72
29 Sep 2016	Horta – Lisbon – Dubai	30	10	100	200	5,6	11,1	0	0	18	72

Date	Amquel (g/m^3)	Carb/Bicarb (g/m^3)	Betadine (ml/m^3)	Ice (kg/box)	O2 (% sat.)		pH		Temperature (°C)		Ammonia (mg/L)	
					Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
1 Oct 2014	0	0	0	0	48	148	5,95	7,89	20,3	21,1	0,0	
15 Oct 2014	25	100	0,1	0	70	273	6,26	7,98	20,0	21,4	0,0	
22 Oct 2014	25	100	0,1	3	94	282	6,32	7,89	14,8	19,3	0,0	
10 Nov 2014	25	120	0,1	4	97	293	6,43	7,97	14,2	19,1	0,0	
12 Dec 2014	25	120	0,1	4	–		–		–		0,0	
17 Dec 2014	25	120	0	4	200+		6,8		15,0		–	
28 Jan 2015	25	120	0,1	4	200+		6,86	7,29	13,0	14,9	0,5	1,5
3 Feb 2015	25	120	0	4	200+		7,00		12,0		–	
5 Oct 2015	25	150	0,1	2	316		8,00		20,9		–	
6 Oct 2015	25	150	0,1	2	200+		7,90		23,7		–	
28 Oct 2015	25	150	0,1	2	200+		–		–		–	
29 Sep 2016	25	150	0,1	2	374,6		8,34		18,4		0,08	

School of Sea Technology, and were then flown to Budapest on a direct flight from Lisbon (3 h).

On the 29th of September 2016, 10 animals were flown from Horta to Lisbon, after which they spent the evening at the airport's warehouse, flying directly to Dubai (6 h) the morning after. Total route times for each delivery are given in Table 3 and these include the complete process, consisting of packing, driving to the airport, export documentation and palletizing, flight per se, import documentation, driving to the end client, and opening upon arrival.

Results

2013: Sea and road transport

The collection, maintenance and transport of all pilotfish was successful, although the introduction to main exhibits containing large predators requires further investigation, as explained further ahead. All 76 *Naucrates ductor* arrived well at their respective destinations (60 throughout the public aquaria listed in Tables 1 and 16 at the Peniche holding station). The only mortalities registered prior to the introduction of pilotfish in aquaria main exhibits were at the Peniche holding station, on the 25th of December (2013), due to the aforementioned power outage, that caused water quality to deteriorate rapidly. Water quality results for sea and road transports, as well as at both Porto Pim Aquarium and Peniche holding stations, are given in Table 2.

During the time the animals were kept in captivity, multiple empirical notes on their behavior were recorded, although an ethogram-per-se was not devised. These observations allowed for the conclusion that these animals are extremely sensitive to handling and are summarized below:

1) Easy development of skin external lesions after rubbing against hard objects or being in contact with human hands.
2) Rapid changes in light intensity induce strong agitation, often causing collision with tank walls or even jumping out of the water.
3) When disturbed by factors such as the two mentioned above, pilotfish proved to be particularly sensitive to parasites, especially *Monogenea* (Cl.), which occurred predominantly in the eyes.

These latter three aspects were counteracted by the following measures:

1) Mandatory use of latex gloves and/or plastic bags with water and/or vinyl stretchers during absolutely all handling.
2) Light dimmers were installed on all premises where *Naucrates* where held. Additionally, great care was taken to not turn lights on/off abruptly, therefore ensuring all light changes were slow and gradual. During all handling procedures done at night, the use of red lighting was found to be quite effective at keeping the fish calm. Sudden movements around the tanks were also avoided.
3) Three minute freshwater baths successfully removed external parasites during maintenance periods at both Porto Pim Aquarium and Peniche holding stations. However, the use of Praziquantel™ (Farma-Quimica Sur, Malaga, Spain) at 10 mg/L over 24 h baths at five days intervals, was preferred since it prevented additional handling.

These animals demonstrated fast and speedy recovery of all external skin lesions, even those with a severe appearance, after treatment with liquid povidone-iodine 10% (1% available iodine), such as the aforementioned treatment consisting of 0.1 mg/L of Betadine™ for 48 h, followed by a 24 h rest and then a second 24 h bath.

Oxygen consumption of pilotfish can be extremely high, which demands for abundant ventilation both in transport and quarantine tanks. Occasionally the animals developed externally visible gas bubbles in the eyes, which often resulted in exophthalmia, most likely exacerbated by *Monogenea* parasites. No treatment was applied to these situations, other than ensuring that no excess nitrogen was present in the water and providing a higher than normal (i.e. > 150%) saturation rate of oxygen, to ensure other dissolved gasses were flushed out of the water.

As to feeding, they showed an extremely voracious appetite. Fed twice per day, there was intense competition between individuals during feeds, which was counteracted by supplying generous doses of food to full satiation of all animals.

With regard to swimming behavior, pilotfish were also observed displaying erratic swimming behavior when no host was present, which occasionally caused them to swim sideways. This behavior was observed frequently although with no apparent consequences to the fish.

Upon introduction to public aquaria main exhibits, all pilotfish regrettably died to predation within less than a week after, despite the fact that the animals were usually introduced inside a floating cage and remained there for two or three days before release. They were mostly preyed upon by *Carcharias taurus, Carcharhinus melanopterus, Triaenodon obesus, Carcharhinus amblyrhynchus* and *Lutjanus sebae*. Against all previous expectation, the transported *Naucrates ductor* were not automatically perceived as 'symbionts' by the sharks and other predators in the exhibits they were introduced to, as was anticipated due to their symbiotic relationship in the wild.

2014–2016: Air transports

The first trial conducted before air transports (Table 3, '1 Oct 2014') revealed a minimum pH of 5.95 and an ammonia concentration of 4.0 mg/L at the end of the 13 h long trial, which strongly suggested the addition of Amquel™ / sodium bicarbonate / sodium carbonate on future trials. Optimal results (i.e. highest minimum pH of 6.43) were achieved with concentrations of 25 / 120 / 120 g/m^3, respectively, on '10 Nov 2014' (Table 3), despite the relatively high ammonia concentration of 1.0 mg/L after 36 h. It was considered, however, that a concentration of 1.0 mg/L after 36 h was acceptable and would not harm the animals, since they would be arriving at their destination after this amount of transit time.

The addition of both ice and Betadine™ also proved helpful in keeping the animals' oxygen consumption and skin lesions, respectively, to a minimum. In light of the results obtained during the trials, the method used for both air transports included all of the buffering, cooling and disinfection agents listed before, including the fact that a bioload of 12.5 kg/m^3 was the maximum allowed per bag.

Despite these actions, the first transport – to Atlanta (Table 3, '17 Dec 2014') - yielded three losses out of five animals, while the second – also to Atlanta (Table 3, '3 Feb 2015') -, with a substantially shorter transit time, yielded one loss out of seven animals, although two more individuals perished the day after arrival. These early losses were attributed to the larger size of the animals (300–500 g), which is corroborated by subsequent results, where the air transport of smaller animals never yielded a single loss again in transit. No necropsies were conducted on the animals lost.

The introduction of the animals to Plymouth's facility was quite successful, with no mortalities in transit, although one individual behaved in a rather erratic fashion and subsequently died approximately three days after arrival. All animals were given a seven-day antibiotic bath at 75 mg/kg bodyweight of fish (oxytetracycline hydrochloride) upon arrival (Aquatet 100%, Pharmaq, Fordingbridge). After the antimicrobial treatment was over, temperature was slowly increased from approximately 16–17 to 22–23 °C, before the animals were moved to an acclimation tank, where they were mixed with golden trevally (*Gnathanodon speciosus*) and snubnosed pompano (*Trachinotus blochii*) with the objective of creating a mixed shoal prior to release to the main exhibit. A large tub was introduced in the acclimation tank and once the whole shoal was inside, they were transferred to the main exhibit, together with a honeycomb whiptail ray (*Himantura uarnak*), which was suspected to predate upon four animals at night. Once the ray was separated from the group, the animals did well until an incident involving lighting caused them to abruptly impact against the tub, which caused every member of the group of 15 to die within the next days. No necropsies were conducted on the animals lost.

The introduction of animals to Budapest, Springfield and Dubai facilities followed standard protocols of acclimation upon arrival and there were no losses during transport, or the days after arrival. Subsequently, however, losses occurred after the introduction of the animals to their respective main exhibits in both Budapest and Dubai, all falling to predation over multiple days.

The 20 animals shipped to Springfield did initially well in quarantine but became infected with *Monogenea* in the eyes a few weeks after arrival. Approximately half were successfully treated with Praziquantel baths, while the remaining were lost. The surviving animals were moved to a new exhibit devoid of any cohabitants. Other schooling teleosts were added a few months after and gradually all pilotfish were lost over refusing to take food or jumping out of the tank, which was attributed to excessive noise from nearby construction works. No necropsies were conducted on the animals lost.

Discussion

The transport regime adopted to move these pilotfish by sea and road was successful on all legs, i.e. by sea over four days and by road over eight days, meaning that no mortalities occurred in transit. Transport conditions were emulated after other transports conducted by the authors, namely for chub mackerel (*Scomber japonicus*) and Atlantic bonito (*Sarda sarda*) (Correia et al. 2011), and also bull rays (*Pteromylaeus bovinus*) and dolphinfish (*Coryphaena hippurus*) (Rodrigues et al. 2013). However, the authors believe a key part of the success of these transports was the fact that these pilotfish traveled alone in their transport tanks and had no other specimens from other species in the same space, which would have potentially altered their behavior substantially. This strategy was adopted since, occasionally - during pre-transport times at the Porto Pim Aquarium -, specimens from other species were placed in the same tank with pilotfish, and these latter ones did not react well to such introduction, displaying erratic and aggressive behavior, such as swimming faster than normal, and hitting the tank walls. This also caused oxygen consumption to peak, which led to additional causes of disturbance in their behavior.

The tank sizes used proved to be enough for the bioload present in the tanks. One other key factor that contributed for the success of the sea and road transports was the continuous oxygen supply to the water, which most of the time was kept above a 150% saturation rate, as seen in Table 2. Oxygen was a key issue to observe because, as stated earlier, these animals display high oxygen consumption rates, as previously illustrated by Weber and Haman (1996). As a result of their high metabolism, it became necessary to keep them well fed, which is difficult when transporting animals by air or road. However, the feeding regime of at least one meal per day had to be kept while on the road. Feeding were therefore made to coincide with the arrival to each institution, to allow for the siphoning out of leftover food and a large water change after. Care was taken to ensure out-flowing water was properly disposed off, using each institution's sewage water treatment system. This protocol ensured ammonia concentration remained close to 0.00 mg/L and pH above 6.90 throughout the 12 day long sea and road trip. The filtration systems used (both chemical and physical) also proved to be adequate but it became clear, early on the trip, that filtration and water changes were not going to be enough to keep water quality satisfactory throughout the trip, hence the previously mentioned Amquel™ and pH buffering cocktails being used consistently, which greatly assisted in keeping water quality optimal.

As far as capture, the authors believe that a simple method of capture, such as the regular hook and line apparatus that was used, is the most effective and sustainable way of retrieving these animals. Transport from the ocean to shore, was improved by using a larger 135 cm round tank with a direct intake of seawater from 2014 onwards, rather than the rectangular recipient used in 2013.

While at the Porto Pim Aquarium, husbandry conditions proved to be ideal and all issues stated in the results section were not attributed to handling issues but, rather, to the extremely fragile nature of the animals' delicate skin. Plastic bags were therefore used on all movements, instead of nets. Despite these measures, whenever external abrasions were visible, Betadine™ baths proved to be very effective and provided full recovery, as previously noted by Udonkusonsri and Noga (2005) also.

Erratic behavior was also observed when sudden light changes occurred, which were easily resolved by slowly lighting the room on or off. This erratic behavior included colliding with the tank walls, jumping out of the tank and vertical swimming. Jumping was prevented simply by deploying a styrofoam plank over each tank. The authors also considered that the erratic swimming (mainly vertical) was due to the absence of a host, making these fish swim more vertically in order to preserve kinetic energy (Takagi et al. 2013) due to the hydrodynamics involved.

Once at the Peniche holding station, the erratic behavior of *Naucrates ductor* was less frequent, since the time spent at the Porto Pim Aquarium allowed for the staff to gain a substantial amount of knowledge on how to keep these animals. In Peniche, several of the issues faced in the Azores were counteracted by adopting simple solutions. For example, the light sensitivity issue was resolved by covering each tank window with a black plastic bag, which was taken off slowly once the lights were on. This allowed for the animals to become gradually adapted to changes in lighting.

Unfortunately, all pilotfish introduced to public aquaria during the sea and road trip, were eaten by predatory specimens living in the exhibits they were introduced to, despite multiple attempts to introduce them slowly, or acclimating them inside a floating cage. It is the authors' belief that this issue may be resolved by introducing the pilotfish simultaneously with other schooling fish, such as mackerel (*Scomber* spp.) or horse mackerel (*Trachurus* spp.), or maybe even introducing them directly by a diver near a host species, such as a large shark. One other option would be to introduce the pilotfish to an exhibit prior to any predator, thus avoiding the distress caused by introducing these animals to an enclosure where potential predators are present and already fully acclimated, most likely displaying a sense of dominance over it.

The one notable exception to the previously mentioned results was Springfield, where the animals did not fall prey to predators but, instead, to parasites and excessive noise, which prompted erratic behavior, often leading them to jump out of the exhibit, even over barriers that were placed to prevent these.

The capture, sea, road and air transport, and husbandry techniques used for *Naucrates ductor* were successful, since these steps involved virtually no mortalities, with the exception of the two initial air transports, which involved animals that were too large. The only mortalities observed were due to external conditions on arrival. Initial results on transport by air fell rather short of expectation, with 60% losses in the first shipment and 43% losses in the second, despite both transport protocols following methods that had yielded very positive results during all trials conducted. The high mortality rate in the first shipment was attributed to the long transit time, while mortalities in the second transport were attributed to the large size of the animals, which grew incredibly fast during their tenure at the Porto Pim Aquarium.

Subsequent transports by air involved substantially smaller animals, which yielded 100% survivorship to all destinations (Table 3), regardless of transit time, which strongly suggests that size – and therefore bioload – is a key aspect in the success of the transport of these specimens inside sealed plastic bags. The smaller size of the animal may also play a part in their survival in addition to a lower bioload, since a smaller size allows for easier swimming.

In conclusion, *Naucrates ductor* is an interesting species, very active, with a very high metabolism, and quite striking to the public eye, especially when swimming along large predators. However, husbandry of this species calls for multiple specific aspects that need to be observed, mostly related to creating a calm and quiet environment, as well as optimal water quality. Animals should also be moved at a very small size, preferably under 200 g, with the addition of an ammonia detoxifier, pH buffering agents, disinfection agents and ice, all of these considered critical for the success of a transport, as well as a short transit time. The critical aspect of the process, however, remains in the acclimation to a large exhibit with predators, which seems to have a lethal effect and must therefore be replaced by an alternative method.

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Compliance with ethical standards Flying Sharks' activities are supervised by the University of the Azores, at the Porto Pim Aquarium, and by the Superior School of Tourism and Sea Technology in Peniche. Both institutions can vouch that Flying Sharks' activities, during the works described in this manuscript, adhered to local legislation on animal welfare and no animals were deliberately harmed during this process.

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