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Packing and Shipping

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Abstract

The aquarium industry as we know it today is greatly influenced by the possibility of transporting aquatic species worldwide. For most of the common marine species in this industry, their natural habitat is often located half a world away from their final destination. The transportation process, however, used to be a synonym for high mortality rates. High standards for collection and premium post-harvest husbandry practices are of paramount importance to achieve success in marine species transportation. Still, and despite the adoption of modern high standard practices, this may not be sufficient to ensure low mortality rates. Transport itself can be highly stressful and physiologically challenging to the organisms and may lead to mortalities. In order to reduce these mortality numbers, numerous techniques have been tested and applied globally and, in some cases, with great success. Some of these techniques are here described in detail.

Keywords *IATA Live Animal Regulations; polystyrene boxes; transport methods; tanks*

26.1 Introduction

Trade in live aquatic ornamental animals for the aquarium industry is a global activity that allows a hobbyist in North America to keep a clownfish from Polynesia in his living room's nano-reef tank, or a public aquarium in Asia to exhibit Barber gobies from South America. Ornamental marine species (corals, other invertebrates and fish) are collected and transported mainly from Southeast Asia, but also increasingly from several island nations in the Indian and Pacific Oceans, to consumers in main destination markets: United States of America (USA), European Union (EU) and, to a lesser extent, Japan (Wabnitz *et al*., 2003).

Although some marine aquarium fish and invertebrates are aquacultured by the industry and hobbyists, most marine ornamentals – over 90% – are from wild-caught fisheries (Chapman *et al*., 1997; Cato & Brown, 2003). Considering that most of these fisheries occur in coral reefs (commonly regarded as amongst the most biologically rich and productive ecosystems on Earth), it is extremely important that marine ornamental

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fisheries are managed in such a way that they are biologically sustainable, do not conflict with other resource uses and keep post-harvest mortalities to a minimum (Wabnitz *et al.*, 2003).

Marine organisms often experience high post-harvesting and transport mortalities. These mortalities typically range from a few to more than 80%, as a result of a combination of the use of cyanide to stun fish, poor capture and handling techniques, inadequate husbandry practices, facilities and transportation, and the inclusion of unsuitable species in the trade (Johannes & Riepen, 1995; Wood, 2001; Sadovy & Vincent, 2002).

Even assuming that good practices of collecting and handling have been adopted, it is important to ensure that all organisms are perfectly healthy and stabilized before packing for transport. The collection process, as well as the organism's introduction to captivity, may cause mechanical abrasions, stress and discomfort, amongst multiple other problems. Therefore a "stabilization period" is critical for the animal to recover from these symptoms and, consequently, maximize its chances of survival during and after transport. This period may differ considerably according to numerous factors, including species resilience and adaptability and seriousness of the lesions on the organism. The adoption of good husbandry practices is crucial and may decrease this stabilization period. Only once the animal is healthy and fully recovered from the harvesting/introduction to captivity/stabilization period factors, should one consider packing it for shipping.

While preparing for shipping, a myriad of details has to be carefully analyzed and various options should be considered in order to transport animals safely for a reasonable cost. One of the first questions to be considered is the container where the animal will travel. For example, transporting a 1 m long Blacktip reef shark entails completely different logistics (and container) than transporting a 4 cm Pacific cleaner shrimp. The shark most likely requires a round polyethylene vat with at least 1500 L of seawater, constant water flow, oxygen supply, and preferably a filtration system, so that the animal may survive 24 h en route safely. The cleaner shrimp, on the other hand, may travel well inside a sealed plastic bag with 1 L of seawater and 0.5 L of oxygen above the seawater. This "closed system" would be enough for the animal to survive a 72 h journey safely. In general, animal transport can be divided into two different categories according to packing option: bag (sealed) transport and tank (open) transport.

26.2 Packing

Transporting live animals is a delicate operation that involves some risk. Poorly planned and executed transport may cause mortalities during transit or during the early hours that follow acclimation at the destination. The stress (both physiological and behavioral) felt by an animal that undergoes transport must always be taken into consideration and all measures that minimize this stress should be adopted in order to reduce (or, preferably, eliminate) mortalities. As such, some useful techniques are commonly used worldwide to reduce animal stress.

26.2.1 Container Size and Shape

The use of an adequate container is absolutely critical since this will be the animal's home during the following hours/days. The container must be of an adequate size for the animal to be transported. The size of the container depends not only on the animal's size and weight, but also on the specific animal behavioral and physiological characteristics. For example, it is possible to transport a certain bioload (kilograms of body weight per liter of water) of Madeira rockfish, *Scorpaena maderensis*, in a sealed plastic bag with 5 L of water and 5 L of oxygen for 48 h. However, it is not possible to transport in the same conditions bioload of Chub mackerel, *Scomber colias*. The behavioral and physiological characteristics of rockfish are completely different from those of the mackerel. The first has a sedentary lifestyle and a relatively low oxygen consumption rate, while the latter is a typical pelagic free-swimming fish that requires lots of space to swim and exhibits high rates of oxygen consumption. In this case, the plastic bag packing would be a good option for the rockfish, but not for the mackerel. For the mackerel, a tank with permanent oxygen supply should be used, as described by Correia *et al.* (2011).

The shape of the container is also very important in terms of animal welfare and comfort. For example, when an animal is transported inside a plastic bag, it is important to ensure that it does not get stuck if the bag folds during the transport movement. In this sense, the plastic bags should be fully inflated and corners must be eliminated. This can be done by using round bottom bags, by taping the corners of the bag or simply by inverting the bag into another bag.

As for a tank transport of, for example, a Dolphinfish *Coryphaena hippurus* – a typical open ocean species – it would be necessary to use a round tank, with no corners, as described in detail by Correia (2001), Young *et al.* (2002), and Correia *et al.* (2008, 2011), to prevent the animal from colliding against the walls while swimming, and with no obstacles, so that the fish can swim freely inside the tank, as suggested by Rodrigues *et al.* (2012).

26.2.2 Water Parameters

When packing an animal, top quality water should be used and its parameters should meet the specific animal requirements. Under a normal situation, water parameters will change during transport, and possibly affect animal welfare. Dissolved oxygen, for example, will decrease during transport; temperature may increase or decrease according to external factors; pH will also decrease over time; ammonia, on the other hand, will increase during animal transport. These are the most important water parameters to monitor during transport as they are both highly likely to fluctuate. These fluctuations can, however, be minimized or even eliminated, which will be described afterwards.

26.2.2.1 Dissolved Oxygen

Oxygen depletion in transport water is caused by animal metabolism and it is a common cause of mortalities in transit. By using an oxygen cylinder and an airline tube while packing, bags can be inflated with oxygen, supersaturating the water (i.e. driving the oxygen saturation rate, in the water, far above 100%), thus eliminating this problem. The amount of oxygen volume added during packing will depend on the species' consumption rate, but usually 1/3 water and 2/3 oxygen is an acceptable ratio for most organisms. As transport progresses and the animals consume the oxygen dissolved in the water, the atmosphere of pure oxygen above the water, sealed inside the bag, will dissolve into the water, which typically drives saturation rates to the high 100s or even 200% over transport.

As for tank transports, one can use the same system as with bags, i.e. fill the tank to 1/3 with water and then seal the tank. Afterwards, a small hole is made in the tank lid, just large enough to fit an airline through which oxygen is pumped until it eliminates the 2/3 of air left in the tank above the water, replacing it with pure oxygen. The inner side of the tank lid may be fitted with an air pump coupled to an air stone, which will diffuse the oxygen into the water progressively. Once the tank is supersaturated, this hole must be sealed with silicone or another isolating material. One other option is to travel with an oxygen cylinder along with the tank, thus providing oxygen continuously through an airline and an air stone.

26.2.2.2 Temperature

Temperature shifts in transport water are common during transit and this happens mostly because of changes in ambient surrounding temperature. It is important, therefore, to keep temperature as stable as possible in order not to jeopardize the animals' wellbeing. This is true for both tropical and cold water species. During bag shipments, putting the bags into polystyrene boxes is a very common technique, as this material offers excellent thermal insulation properties, while also providing adequate mechanical protection. Adding newspapers or plastic bags inflated with air to the polystyrene box, between the bags with animals, is also common and efficient both for thermal and mechanical protection. Newspapers will also absorb any residual water that may be on the outside of the bags. One other common technique that is used to maintain temperature of transport bags is the use of ice (or heat) packs inside the polystyrene transport box, depending on the temperatures expected to be experienced during transit and animal requirements. If temperatures during transit are expected to be much lower than water temperature in the transport bag, heat packs can be used. On the other hand, if temperatures during transit are expected to be much higher than water temperature in the transport bag, the use of ice packs is advisable.

Controlling water temperature in tank transports is not practical with any of the aforementioned techniques, since none of them is effective for a large volume of water, typically above 1000 L. On the other hand, in large water volumes, temperature oscillations are much less likely to occur, unless tanks are exposed to extreme outside air temperatures for a long time. As long as this is avoided, temperature oscillations should not constitute a significant problem in tank transport.

26.2.2.3 pH and Ammonia

During transport, water pH will gradually decrease, whereas ammonia will increase, as a result of carbon dioxide buildup and the release of nitrogenous waste and miscellaneous stress-related metabolites. Both of these tendencies need to be counteracted through the use of filtration (only possible in tank transports using filters) and/or chemical supplements. Ammonia buildup may also be minimized by fasting the animals before transport.

26.2.3 Filtration

Water filtration is possible, and advisable, during tank transports. As described by Correia (2001), Young *et al.* (2002), Correia *et al.* (2008, 2011), filtration units can be used to help maintain water quality during transit. These units can be relatively simple,

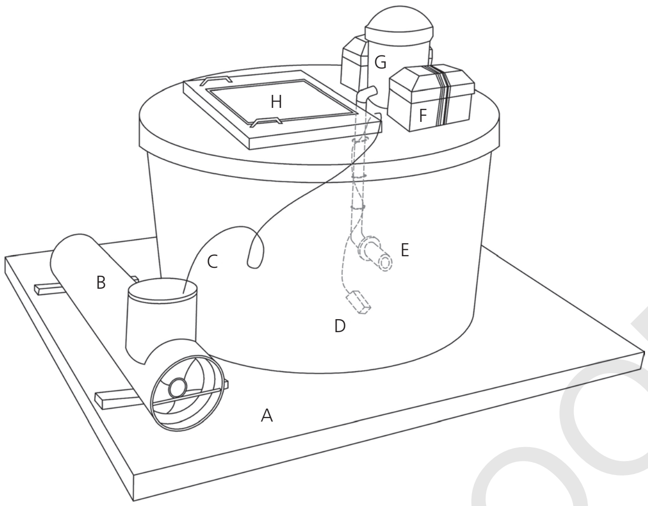

Figure 26.1 Transport tank for live fish. (A) Wooden, or plastic, pallet; (B) Stainless steel cylinder to hold oxygen cylinder (inside); (C) Airline tubing, connected to pressure regulator, in turn connected to oxygen cylinder; (D) Air stone (inside the tank); (E) 12 Volt bilge pump; (F) 12 Volt dry-cell battery; (G) Filter unit (usually laminated cartridge paper and one mesh bag with activated carbon inside); (H) Inspection and access window.

consisting of containers with laminated cartridge paper and one mesh bag with activated carbon. But more complex units have been used, such as foam fractionators (Rodrigues *et al.*, 2012), which provide additional and continuous removal of organic matter from the water, thus eliminating the source of pH drops and ammonia buildup. All these systems are, as described by the authors, powered by 12 Volt dry-cell batteries (i.e. appropriate for air-travel), wired to bilge pumps (Figure 26.1).

26.2.4 Chemical Agents

The control of pH can be achieved by the use of buffering agents, such as the tribuffer described by McFarland & 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 NH_4^+) may be removed with the assistance of quenching agents such as AmQuel® ($HOCH_2SO_3$) (Novalek, Inc., Hayward, CA, USA) which binds to ammonia and transforms it into nontoxic aminomethanesulfonate ($H_2NCH_2SO_3^-$) and water. The addition of these three agents (AmQuel®, sodium bicarbonate and sodium carbonate), according to the formula 20/50/50 g per 1000 L (Correia *et al.*, 2011; Rodrigues *et al.*, 2012) to the water while packing, and before adding the animals, will help prevent pH drop and ammonia buildup. Other types of chemical agents such as sedatives and other anesthetic agents can be used in transport; however, their influence will be on the organisms instead of treating the water. The stress in the animals (especially fish) caused by the transport process, many times in crowded conditions, can be reduced with chemical anesthetics, by producing a light sedation.

Various products have been widely tested for fish sedation (e.g. Quinaldine, Benzocaine, Tricaine methanesulfonate, 2-phenoxyethanol, clove oil) (Munday &

Wilson, 1997; Neiffer & Stamper, 2009) but the most commonly used in transports is Tricaine methanesulfonate, commonly known as MS222. This chemical is effective for most species of fish, but concentrations to produce light sedation vary for each species and depend on size and weight of the animals (Neiffer & Stamper, 2009).

26.2.5 Fasting

One other commonly used technique to prevent ammonia buildup is animal fasting prior to shipping. Not feeding the animals that are about to be packed for shipping is an effective way to reduce excretions and, therefore, reduce ammonia production. This fasting period depends on the species to be shipped but, generally, 48 h is considered adequate for most species. As a general rule, the smaller the animal, the shortest the fasting period needs to be. Also, if the animal is a constant swimmer (e.g. sardine) or a relatively sessile animal (e.g. scorpionfish), different fasting periods should be considered. The sardine should undergo a shorter period of fasting than the scorpionfish since the first will spend its energetic reserves faster than the latter. Usually, tropical species also fast for shorter periods than closely related cold-water species, since the first usually have higher metabolic rates and spend energy faster (e.g. tropical and cold-water *Anthias* spp. can have remarkably different habits and metabolic rates). It is important to remember that this fasting period will continue through transit and may be extended for some time after arrival to the destination. On arrival, the animals may not feed for various reasons such as stress, unfamiliar environment, and new food items, amongst others. Therefore, all measures should be taken so that the animal does not go without food for too long, thus compromising its health.

26.3 Shipping

26.3.1 Road, Air, Sea Transport

Shipping live marine animals may be done by air, road or sea. All three methods entail common concerns, highlighted in the previous pages of this chapter, but they also host specific aspects that will be addressed individually next.

26.3.1.1 Road Transport

Moving live marine animals by road usually involves long trips, typically over 12 hours. During this period of time, water quality deteriorates, as described above, but road transports allow for water changes (i.e. blowdown) to be performed in transit. It is therefore advisable, if possible, to pack extra clean water and pumping equipment (i.e. pump, adequate length hose and power source, such as batteries). One should note that saltwater is a highly corrosive substance, so care should be taken during water changes, specifically while saltwater is being drained from a transport tank. This wastewater should be channeled, with a hose, to a sewage intake whenever possible. Dumping saltwater onto a non-regulated destination could, not only cause harm or malfunction to the equipment that comes into contact with such saltwater, but may also land the person a heavy fine, with unwanted and unnecessary delays.

Moving tanks by road also offers unusual high level of vibration, which needs to be addressed by ensuring that all equipment is securely fastened. Should temperature constitute a concern, trucks equipped with a heater (or chiller) unit should be used. Other additional concerns come in the form of simple logistics that may prove to be extremely important. For example, transport tanks are typically loaded on top of pallets, which are then loaded onto a truck's flat bed. When loading, it is important to learn whether the truck will be offloaded from the side or the back, so that the pallets are oriented in the correct way. Note that both tanks and pallets are easily moveable by hand when empty, but become virtually impossible to be moved by hand when filled with water. The proper orientation is therefore critical, the penalty for neglecting this aspect being the offloading of the fish from the tanks by hand while they are still loaded on the truck, and carrying the heavy bags, or nets, with fish.

Road transport offers multiple advantages, the most significant being their relatively lower cost compared to air transport. Additionally, road transport allows for filtration units to be wired directly to the trucks' power supply, which means no batteries are required to run the filtration pumps wired to the transport tanks. Alternatively, batteries may still be coupled to all filtration units and be charged on the road during transport, ensuring they are fully charged upon offloading the truck. This may prove extremely convenient if, for example, the tanks have to be left unattended for some time. However, great care must be taken to ensure both voltage and amperage are compatible. For example, most bilge pumps used in transport run on 12 Volts DC current, while some trucks have 24 Volts DC batteries. Wiring one to the other would result in burned pumps. The same concerns need to be observed for both amperage (measured in Amps) and frequency (measured in Hertz or cycles/second) to avoid equipment damage and/or, loss. In addition, voltage inversers may be coupled to the filtration system, converting the 12 Volt DC power of the vehicle to 110/220 Volt AV, which allows for the use of standard 110/220 Volt pumps and therefore more powerful and stable filtration, since the use of 12 Volt batteries is unfortunately coupled with a decrease in charge over time, which causes filtration to lose momentum and requires constant fine tuning of the equipment, particularly protein skimmers. Care should be taken to use "pure wave" inversers which, albeit more expensive, provide for a more stable and constant flow of voltage, and therefore a more smooth operation by all pumps and less need to constantly fine tune them.

26.3.1.2 Air transport

Air transport offers speed to reach the destination, but comes with two often deterring disadvantages: cost and regulations. Air-travel is indeed substantially more expensive than road travel and also entails a rather heavier administrative burden. For example, the use of compressed oxygen cylinders on board aircrafts is highly controlled, as it is considered a "Dangerous Good". Oxygen may still be used but a "Dangerous Goods" declaration needs to be filled out and the airline will have to request multiple permits from official aviation agencies in all countries over which the aircraft will fly.

Another major concern while flying transport tanks is that absolutely no leaks are allowed inside an aircraft, particularly of saltwater, which is not only extremely corrosive but highly conducting as well. There is a "famous" (or "infamous") story in the industry where a leaky tank, during take-off, allowed for some saltwater to seep into the avionics compartment of a cargo Boeing 747, triggering all sorts of alarms and malfunctions, forcing the crew to do an emergency landing immediately after take-off. This means that the shipper using transport tanks and/or polystyrene boxes, needs to ensure that not one single drop of saltwater will leak during transit. Leak-proofing transport

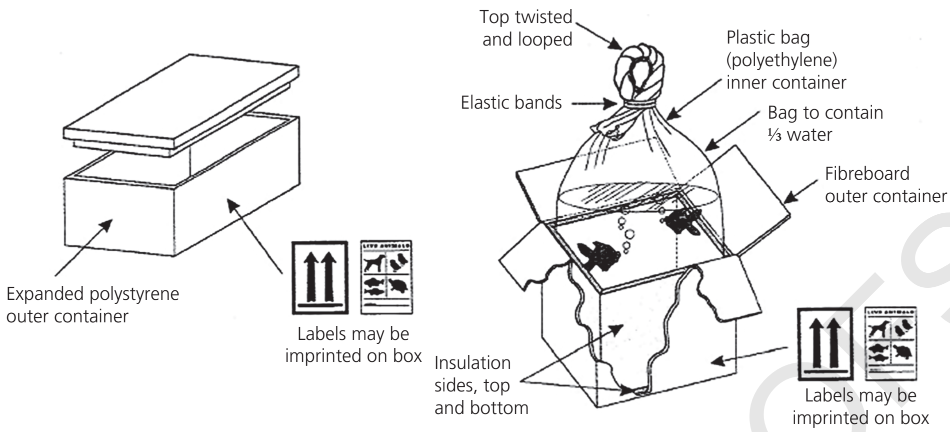

Figure 26.2 IATA (International Air Transportation Association) Live Animal Regulation number 51, for transporting live fish inside plastic bags, which travel inside polystyrene boxes that, in turn, must travel inside a cardboard box.

tanks basically involves a generous dose of sealant material (such as silicone) and a large number of nuts and bolts (typically 20 cm apart along the edge) securing the lid to the tank itself. When moving polystyrene boxes the bags inside need to be doubled, sometimes even tripled, often with newspaper, or another insulating material, in between bags. The polystyrene box, with the bags inside, is then placed inside a cardboard box, for added thermal and mechanical insulation, as well as safety. These requirements are described in great detail under "Live Animal Regulations" section 51 of the International Association of Air Transport guide for moving live animals (Figure 26.2).

Temperature on board commercial aircrafts' cargo holds is usually kept stable at 15 degrees Celsius, which is adequate for the vast majority of specimens, and both heat, or ice, packs may be included in the boxes, as described before. When flying cargo aircrafts, the crew often has the ability to control the cargo hold's temperature, enabling attendants who fly with the animals to request their optimal temperature.

26.3.1.3 Sea Transport

Moving live marine animals by sea offers two major advantages: cost and water. Cost of maritime freight is substantially lower than air, or even road. Traveling on a vessel allows for continuous, or frequent, water changes provided that the water being navigated offers enough quality and parameters for it to be used with the animals being moved. Sea freight is typically only used for transport tanks, as moving styrofoam boxes is easily done for a reasonable cost by air or road. Major concerns, while moving transport tanks by sea, involve safety of both the animals and persons attending to the animals' needs in transit. All parts and equipment need to be secured properly to avoid movement during rough seas. People should also take great care while moving around on a large ship, which even during flat seas, offers a multitude of possibilities for injury.

Temperature control is often not available, unless temperature controlled containers are used during the trip. Another potential disadvantage in sea-freight is "time", as oceanic trips typically last longer than 48 h with extremely limited opportunity for procuring supplies or materials. Great care therefore needs to be taken upon boarding the vessel, as every single item that may possibly be required must be shipped with the transport tanks and their animals. Sea freight should therefore be limited to species that may handle 48+ h in transit, if not much more.

26.3.2 Administrative Issues

Shipping live animals to international destinations involves a myriad of support documentation, described next.

26.3.2.1 CITES

CITES (Convention on International Trade in Endangered Species – www.cites.org) acts along the borders and is controlled by customs agencies worldwide. As such, CITES licenses and declarations are not required for domestic or intra-European transports, but are required when borders are crossed to, or between, third countries. CITES lists animal and plant species in appendices I, II and III, depending on the level of protection from over-exploitation each species is attributed. CITES listed species require a license issued by CITES agencies in both the origin and destination countries. Species not listed in CITES appendices do not require a license but still require both an export and import declaration issued by the origin and destination country's agencies, respectively.

26.3.2.2 Veterinarian Certificate

Most countries require a veterinary certificate upon importation but be aware that veterinarian certificates have multiple formats in different countries. When applying for a veterinarian certificate, the exporting country's governmental veterinarian agency will request advice from their counterpart in the importing country, then issue the certificate according to the importing country's agency requirements. Be aware that often governmental veterinarian agencies take an extremely long time to communicate amongst each other, and direct communication between the exporter and importer might be advised regarding this issue. Most veterinarian agencies will require a veterinary declaration, issued by a certified veterinarian doctor, before the governmental official document is issued.

Intra-European shipments require an "International Transport Permit", which is issued by an online system known as "TRACES", which stands for "Trade Control and Expert System". This permit may be issued by the exporter directly from the Europa.eu website (http://ec.europa.eu/food/animal/diseases/traces/index_en.htm), although an official seal is often required by that country's governmental veterinarian agency. Once again, the seal is not issued until a veterinarian declaration, signed by a certified veterinarian doctor who has physically inspected the animals, is provided.

26.3.2.3 Certificate of Origin

Most non-European Union countries require a Certificate of Origin from the exporting party, which should be checked with the importing party. These Certificates are typically issued by the exporting country's Chamber of Commerce.

26.3.2.4 Customs Documents

Customs documents are required for all non-European Union shipments and come in multiple formats. We therefore strongly advise all those exporting, or importing, live animals, to/from a non-European Union country to procure the assistance of a customs agent who is certified to handle all customs related documents.

26.4 Conclusions

Shipping live marine animals is no easy task, but it is also perfectly achievable for most animals with a reasonable degree of effectiveness, provided all logistical and administrative aspects are addressed adequately. Communication between both exporting and importing parties is crucial to ensure all required documents are taken care of in a timely fashion. Logistic concerns then follow, with choosing the packing method that ensures the highest standard in animal care, taking into consideration the behavior, and special needs, of all specimens that will travel. Length of the trip is a key aspect to be factored in during the decision making process and adequate weighing of all variables will no doubt ensure a positive outcome and no mortalities in transit, or after.

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