Reducing Ich Infections In Fish Arriving At The Beastiary Pet Shop

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Abstract

This report frames the opportunity for reducing the number of fish that come to The Beastiary Pet Shop with Ich flare-ups caused by the stresses of transportation. The scope of this request for proposal (RFP) has been narrowed down to freshwater, tropical, docile ornamental fish transported by road from Ontario and Montreal as they are the most common at The Beastiary. Ich is present in all aquariums but is only triggered when the fish is stressed. The Beastiary is forced to quarantine all newly arrived fish for several weeks, as Ich is highly contagious after it is triggered. Furthermore, Ich symptoms may only be visible 1-3 weeks after arrival [1]. Ich causes several health issues within affected fish, such as lethargy, loss of appetite and death if left untreated.

As the opportunity focuses on the flare-ups of Ich and stress in transported fish populations, fish are the major stakeholders. The Beastiary's employees and owners also prioritize animal and fish care and have a vested interest in the well-being of fish during transport. The customers of the shop and fish owners, in general, are also prominent stakeholders in the opportunity. Secondary stakeholders include the transportation and mailing companies transporting the fish, and veterinarians that treat Ich in severe cases of the disease.

The prominent objectives prioritize the safety and mental well-being of the fish. The requirements cover temperature, water hardness, pH regulation, monitoring oxygen, and mitigating ammonia levels in the water. Additionally, lighting and turbidity of the water can greatly impact fish, making them more prone to stress, and degrading their immune system [2]. Any animal testing must be done with authorization or supervision from professional animal care experts and with proper equipment and doses that do not hurt the fish. Furthermore, the objective is to develop a device that is sustainable and can carry the same number of fish as in regular fish transportation methods. Consequently, it is required they limit the emission of CO_2 per unit mass material used in production, and have a low assembly-to-time ratio.

Existing designs for fish transport include plastic bags, breather bags, StressGuard, and clove oil. All of these solutions have their purpose and strengths, however, they do not meet all requirements in the RFP. The improved bag designs fail to meet water quality requirements such as pH, while also not being sustainable. The StressGuard and clove oil both reduce the stress of the fish but fail to meet the requirements for maintaining water quality.

[1] "CIR920/FA006: Ichthyophthirius multifiliis (White Spot) Infections in Fish." Accessed: Feb. 16, 2025. [Online]. Available: https://edis.ifas.ufl.edu/publication/FA006

[2] W. Helmut, H. Daniela, G. Manuel, and K. Gunter, "Practical and legal aspects of transporting live fish," Bavarian State Research Center for Agriculture (LfL), Vöttinger Strasse 38, 85354 Freising-Weihenstephan, Germany. Accessed: Feb. 15, 2025. [Online]. Available: https://www.aquaculture-welfare-standards.net/wp-content/uploads/2021/06/ita-transporting-live-fish-practical-and-legal-aspects-copyright-permission-by-lfl.pdf

1. Introduction

This request for proposal establishes an opportunity to lower the rate of Ich flare-ups in fish caused by stress during road transportation to The Beastiary Pet Shop. Ich is always present within aquariums, however, left untreated after a flare-up induced by stress it may spread to other fish and result in death. Ich is often triggered in transit due to the stresses of a new environment and careless handling of the fish. Those arriving with the disease must be quarantined for a few weeks before being sold. This report details the impact of Ich on fish transport at The Beastiary and the requirements to address the conditions necessary for the fish and facilitate a user's experience with the design.

2. The Beastiary

The Beastiary Pet Shop, located in Cabbagetown, sells a variety of exotic species to regular customers and hosts terrarium workshops and birthday parties. The vast majority of employees interviewed expressed their passion for wildlife and animal care, as well as their work in animal conservation. Furthermore, the Beasitary demonstrates strong communication; the team encountered owner and employees Josh, Jerry, James and Eric, they were extremely forthcoming and responsive to queries. They also prioritize sustainability and integrity in their work, with set policies to ensure pet care standards are maintained. The community contains a Quarantine Room (Figure 1) in the basement, where newly arrived species receive specialized care. Many have concomitant aspects related to their work at the pet shop, from owning and caring for exotic pets domestically, to organizing and maintaining farms for supply.

Ich is the primary disease that occurs among newly-transported fish populations. Many fish at the shop are transported in bags and deli containers from Ontario and Montreal. They are first brought to the Quarantine Room to acclimate to the new aquarium conditions. Here, employees ensure any diseases, such as Ich, can be treated and prevented from further infecting other fish at the shop.

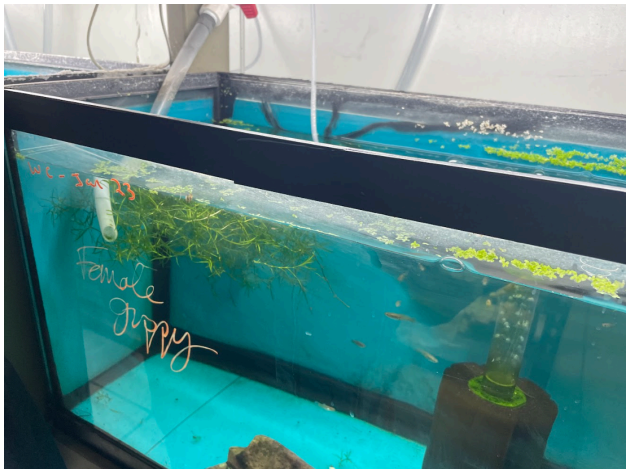


Figure 1: An aquarium tank in the Quarantine Room.

3. Onset and Prognosis of Ich

Ichthyophthirius multifiliis is the primary protozoan responsible for Ich. The disease is marked by "the presence of small white spots on the skin and fins" and can cause a variety of symptoms ranging from irritation and increased mucus, to loss of appetite and weakness [1]. An employee at the Beastiary noted that the protozoan is always present throughout aquariums, but the disease is exacerbated through "flare-ups". It starts as "trophont" and withstands chemical treatment. Following feeding on the epidermal cells of the fish, the trophont leaves and becomes a "tomont", forming a cyst on the fish that then breaks off and attaches to surfaces in the environment. The tomont divides to form up to 1024 "daughter" parasites (tomites) to infect other species and become "theronts", repeating the cycle. [1]

Figure 2: Characteristic "White Spots" indicative of Ich caused by the protozoan *multifiliis*

Source: Adapted from [2]

The primary cause of these eruptions is the onset of stress [3]. Acclimation is capable of preventing the onset of flare-ups [3]. However, this only occurs in the Quarantine Room after being transported to the Beastiary, and there is no mechanism to prevent such flare-ups during transportation itself. Thus, many fish develop the onset of Ich flare-ups when they arrive and must receive treatment consisting of increasing the water temperature to speed up the life cycle of the protozoan and specified medication, such as Malachite Green. If left untreated, Ich "may result in 100% mortality" [1].

Ideally addressing the opportunity would directly measure the number of fish with Ich flare-ups after applying the device and compare it with the typical rate. However, this report is designed with the goal to minimize the amount of animal testing that occurs. Instead, the major objective of this opportunity to reduce Ich flare-ups is instead determined by proxy testing. The focus is on the effect of the environment that the fish experiences on its stress, and by consequence Ich.

4. Values of Integrity and Sustainability

Our team's core values are integrity and sustainability. Individually we all noted these qualities within our position statements as being important to our experiences with engineering design. Furthermore, several team members have experiences with green initiatives, influencing our value of sustainability. We define integrity within our team as a commitment to sticking to our team norms to form stronger unity when faced with challenges. We admired the integrity we observed at The Beastiary, and their commitment to animal health and care although they have yet to become profitable. We elected to work with Ich caused by fish transportation as it reflected The Beastiary's commitment to animal health and integrity in standing by proper treatment of the fish. Furthermore, there are little to no sustainable methods of fish transportation, another aspect that prompted our interest.

5. Defining Scope

The scope of the opportunity has been constrained due to the wide range of fish sold at The Beastiary, the variety of transportation methods, and the responsibilities of the engineer.

5.1 Scoping Fish Species

Our opportunity focuses on improving the transportation of freshwater, tropical, and docile ornamental fish to reduce stress and flare-ups of Ich. This opportunity specifically targets:

Freshwater Fish

There are two types of Ich: *Ichthyophtirius multifiliis* (freshwater Ich) and *Cryptocaryon irritans* (marine/saltwater Ich) from two different parasites [4]. Both saltwater and freshwater Ich cannot survive in the water conditions of the other parasite, and therefore they both have different treatments. Saltwater Ich is more fastidious than freshwater Ich, making freshwater fish more vulnerable to infection [5]. Additionally, freshwater fish are more popular among fish owners and pet shops, making it the primary focus [6].

Tropical Water Fish

Most fish at the Beastiary Pet Shop and among most fish owners are tropical fish, requiring water temperatures around 72ºF (Appendix A). The treatments and requirements for tropical fish and cold water fish vary, influencing our choice to narrow our scope.

Ornamental Fish

Most pet fish sold at the Beastiary are ornamental and 'schooling fish' [7]. To minimize stress, they are placed in groups with at least six fish [7] and are transported in large volumes of

water. However, some types of fish, like the *panda garra,* are hostile towards their own species [7]. To avoid injury from conflict between fish, we are focusing on docile ornamental fish.

5.2 Scoping Transportation Methods

We are particularly focusing on the transportation of fish by road, using automobiles and trucks, to The Beastiary from throughout Ontario and Montreal (Appendix E). The longest expected travel time for the fish is six hours, as the furthest location they source fish from is Montreal, specifically MSR Imporium Canada.

5.3 Scoping Typical Use

Designs may function under the assumption that the user will complete the procedures necessary before and after transporting the fish. The following processes may be assumed to be the responsibility of the user:

- Acclimation of the fish after arrival [3]
- Pausing the feeding of the fish one to two days before transportation [8], [9]
- Filling the device with water with healthy concentrations of chlorine, iron, and other necessary ions as detailed in Figure 3 [10]
- Packaging designs with fragile components safely and with proper orientation and padding [11]

Parameter	Aquarium Range	Natural Range
pH	5.5 - 9.5	5.5 - 9.5
General Hardness	4 - 8 dGH	0 - 18 dGH
Carbonate Hardness	4 - 7 dGH	0 - 10 dGH
Temperature	**68 - 82 degrees F**	**45 - 82 degrees F**
Ammonia	**0 ppm**	**< 0.1 ppm**
Nitrite	**0 ppm**	**< 0.1 ppm**
Nitrate	**0 ppm**	**< 5 ppm**
Phosphate	**< 0.05 ppm**	**0.005 - 0.05 ppm**
Copper	**0 ppm**	**10 ppb (3)**
Iron	**0.1 ppm**	**0.5 - 1 ppm (4)**
Potassium	**5 - 10 ppm**	**0 - 20 ppm**
Chlorine	**0 ppm**	**1 - 100 ppm (5)**
Salinity	**0 - 0.1 ppt**	**0 - 0.5 ppt (1)**
Dissolved Oxygen	**5 - 15 ppm**	**5 - 15 ppm (1)**
Carbon Dioxide	**< 5 ppm (2)**	**5 - 20 ppm (1)**

Figure 3: ”Healthy” ion concentrations in aquariums for freshwater fish.
Source: Adapted from [10]

6. Stakeholders

Several communities, from breeders to customers, are impacted by Ich outbreaks in fish. For instance, fish owners involved run the risk of spreading Ich throughout their other tanks, infecting the other fish in their possession.

6.1 Primary Stakeholders

Fish

The fish that will be transported are the most direct stakeholders for this opportunity. The stress responses of the fish serve as a major metric that informs whether the design has succeeded in fulfilling the primary need of the opportunity.

The Beastiary Owner and Employees

The Beastiary employees and owners value the well-being of the fish. However, they must also consider the priorities intrinsic to running a viable business. Reducing the number of fish that come into the pet shop with Ich will allow The Beastiary to sell the fish they receive faster after arrival. Customers will also receive healthier fish, aligning with the value of animal care the shop espouses and improving their reputation. Furthermore, it would clear space in the shop, reducing the number of quarantine tanks required for incoming fish.

The Beastiary Customers

Customers of the Beastiary value receiving high-quality healthy fish. Less fish coming in with Ich would mean being able to purchase newly arrived fish sooner after their arrival.

Fish Breeders

Fish breeders value optimizing the number of fish in a container to maximize profits, while ensuring they are in good health to avoid unnecessary refunds for fish that are dead on arrival. [9]

Fish Owners

Fish owners desire a method by which they can move their fish between locations or tanks, while keeping their fish safe. [12], [13]

6.2 Secondary Stakeholders

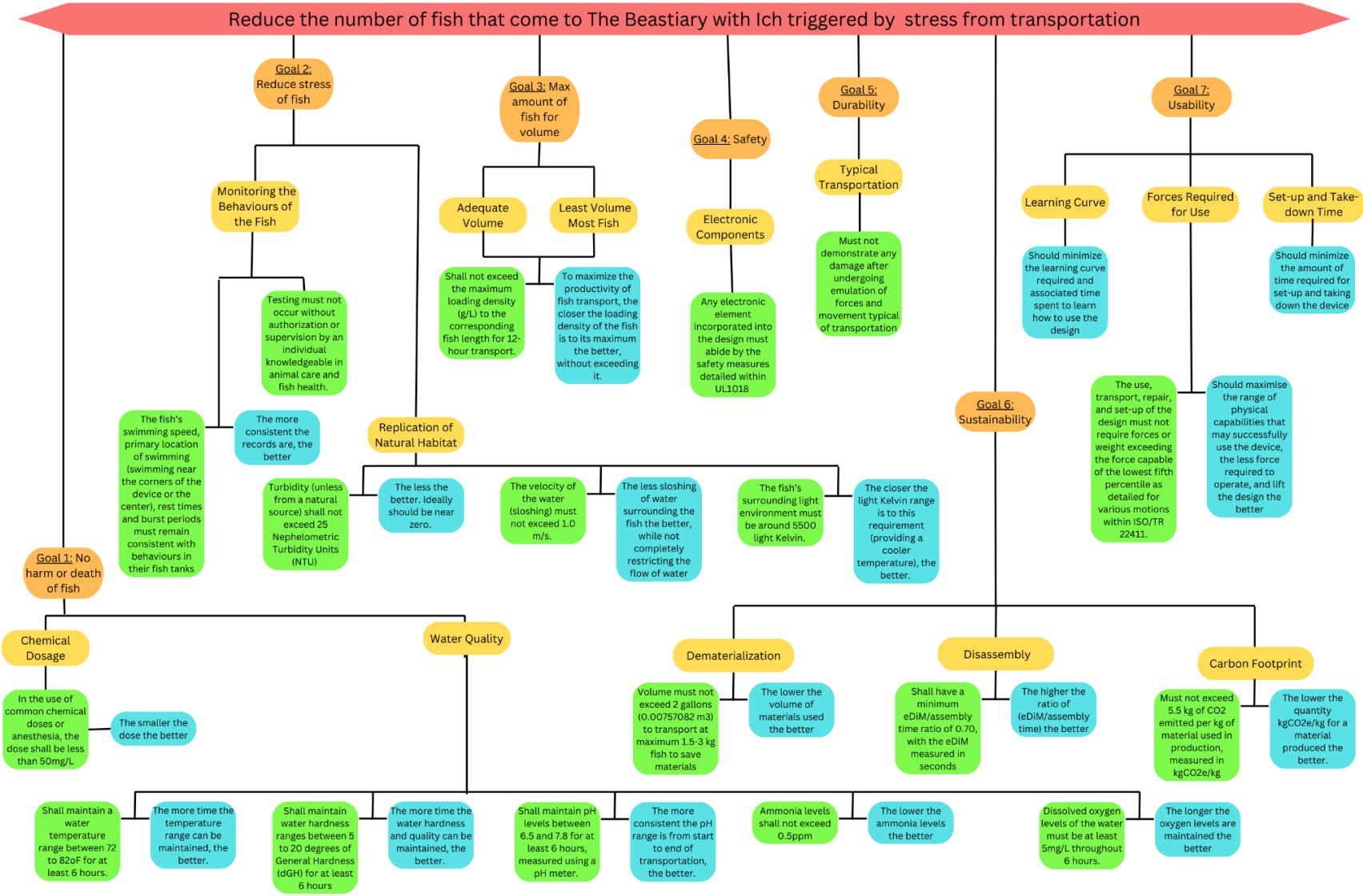
Transportation and Mailing Companies

Fish are typically transported by truck or car by various shipping companies, oftentimes unlabeled as fish. The companies involved in the actual transportation of orders of fish from the providers to the customers are most concerned with the logistics of transporting the fish and their holding containers. These include the weight, shape, and fragility of the containers, due to their liability in the situation where any damage is incurred. [8], [11]

Veterinarians

Less fish with Ich will need to be treated by professional vets in the case where the fish's conditions are too severe for the owner or pet shop to treat. [1]

7. Requirements Graphic



8. Requirements Framework

The major need for the opportunity is to reduce the stress experienced by fish during transportation to decrease the likelihood of Ich flare-ups. The following goals, ordered by highest to lowest priority as informed by stakeholder and team values, denote the qualities of the ideal design.

Requirement	**Evaluation Criteria**	**Metric**	**Justification**
Goal 1: The device shall not damage or harm the fish ***Objectives*** ***i) Water Quality:*** *Ensure the water quality is set and maintained according to the needs of the fish. This includes temperature regulation, water hardness, salinity, pH, ammonia and oxygen levels.* ***ii) Chemical Dosage:*** *Shall ensure that the fish are not surrounded by or in direct contact with lethal doses of chemicals.*			
1.1: Shall maintain a water temperature range **between 72 to 82ºF** for **at least 6 hours.**	*The more time the temperature range can be maintained, the better.*	Conduct the package insulation test as detailed within ISO 22982-2:2021 [14]	Based on information from the Beastiary Pet Shop (Appendix A), 72-82ºF is the optimal temperature for most tropical freshwater fish. Most fish are transported from local shops throughout Ontario and Montreal, which can be approximated to a 6-hour drive, serving as a basis.
1.2: Shall maintain water hardness ranges **between 5 to 20 degrees** of General Hardness (dGH) for **at least 6 hours**	*The more time the water hardness and quality can be maintained, the better.*	Measured using water strips or EDTA chelating agents, noted by Government of Canada [15]	Based on information about ideal water hardness for fish specifically at the Beastiary Pet Shop (results in Appendix B), and the Government of Canada on proper water testing procedures.
1.3: Shall maintain pH levels **between 6.5 and 7.8** for a**t least 6 hours**, measured	*The more consistent the pH range is from start to end of*	Measured using a pH meter or pH strips. It should follow the same testing	Based on information about the ideal pH range for fish at the pet shop (results in Appendix C),

using a pH meter.	*transportation, the better.*	procedures for a recreational swimming pool, outlined in [16]	fact-checked from [17] Swimming pools require a healthy pH between 7.4 to 7.8 at all times[16], which overlaps with the pH levels needed for freshwater fish, making it a reference point.
1.4: Ammonia levels shall **not exceed 0.5ppm**	*The lower the ammonia levels the better; ideally, ammonia levels should be near 0ppm*	Measured using Ammonia Test Strips, the procedure highlighted from [18]	As stated by [10], while fish are fasted before transportation, they often release 'white stringy fish poop' which like all organic waste in fish releases ammonia as it tries to decompose. [19] Additionally, there are types of ammonia; ionized (non-toxic) and un-ionized (toxic). As pH increases, ammonia becomes un-ionized, harming fish.
1.5: In the use of common chemical doses or anesthesia, the dose shall be **less than 50 mg/L**	*The smaller the dose the better.* *Natural sources are preferred over chemical sources for the health and stress of the fish. [20]*	Measuring proper administration of TMS (the most common anesthesia for fish), consult the Canada Department of Oceans and Fisheries Animal-User Training Template [21]	From [20], anesthesia in fish causes prolonged stress as it alters various hormone levels. If the water used to prepare is not the same source as the tank itself, it can create a temperature shock for the fish, stressing and potentially killing the fish.
1.6: Dissolved oxygen levels of the water must be at **least 5 mg/L throughout 6 hours.**	*The longer the oxygen levels are maintained the better*	Measured using The Winkler Method. It should follow the procedure from [22]	The FAO on live fish transport recommends at least 5 mg/L of dissolved oxygen to prevent it from becoming a stress factor. [23][24]

Goal 2: The device shall reduce stress levels in fish ***Objectives:*** ***i) Replication of Natural Habitat:*** *to reduce stress, the fish shall feel they are in their natural habitat. This includes lighting, turbidity, water flow and reducing sloshing during road transportation.* ***ii) Monitoring the Behaviours of the Fish:*** *the fish should maintain consistent behaviour from when they are in their fish tanks.*			
2.1: Turbidity (unless from a natural source) shall **not exceed 25** Nephelometric Turbidity Units (NTU) [25]	*The less the better. Ideally should be near zero.*	Measured using a turbidity meter and as detailed by the United States Protection Agency [26]. Units are in NTU.	Turbidity is the clarity and cleanliness of a liquid. It indicates how much matter is in the water, preventing light from travelling through. High turbidity is a sign of poor and dirty water conditions, which are harmful and stress fish out.
2.2: The velocity of the water (sloshing) must **not exceed 1.0 m/s.**	*The less sloshing of water surrounding the fish the better, while not completely restricting the flow of water*	Using any of the methods detailed by the FAO from [27]	Under strong water flows and currents, fish get stressed and often caught, damaging the tissue. However, no water flow within the water the fish is swimming in also damages the well-being of the fish. [28]
2.3: The fish's surrounding light environment must be **around 5500 light Kelvin**.	*The closer the light Kelvin range is to this requirement (providing a cooler temperature), the better.*	Measured using the Kelvin colour temperature scale or Kelvin colour meter. [29]	Fish prefer natural daylight and environments that mimic their natural surroundings. Natural sunlight at noon provides 5500 Kelvin of light and is said to be ideal for fish [30]
2.4: The fish's swimming speed, primary location of swimming (swimming	*The more consistent the records are, the better*	Tracked and measured using tri-axial accelerator biologgers, with	When many of the fish become stressed and tend to hide near the corners of the tanks (Appendix E), their swimming speeds and

near the corners of the device or the center), rest times and burst periods must remain consistent with behaviours in their fish tanks		procedures detailed in the Marine Ecology Progress Series [31]	respiration change. These fish may require more rest or may have less endurance when swimming, and it is important to note how their behaviour changes from when they are in the fish tank to a transportation device.
2.5: Testing must be authorized or supervised by an individual knowledgeable in animal care and fish health.		Any testing must abide by the conditions detailed within ISO 10993-2:2022 [32].	Any animal testing must be conducted with due diligence and observance of animal ethics. Efforts should be taken to avoid animal testing that may be replaced by secondary research and to reduce any stress the fish may experience. Professional and knowledgeable animal care can lower the likelihood of shock and damage faced by the fish.
Goal 3: The device shall host a maximum amount of fish for an adequate volume ***Objectives*** ***i) Adequate Volume:*** *Should ensure fish has the necessary volume of water to move around and not be stressed.* ***ii) Least Volume Most Fish:*** *Assuming the fish has enough volume, maximize the amount of fish in the container.*			
3.1: Shall not exceed the maximum loading density (g/L) to the corresponding fish length for 12-hour transport. Specific information is found in Appendix D.	*To maximize the productivity of fish transport, the closer the loading density of the fish is to its maximum the better, without exceeding it.*	Using the expected total mass of fish and water volume for the container, calculate to see if it exceeds the loading density.	Based on information from a journal article by the University of Arizona on ornamental fish transportation. Due to information listing 1 or 12 hrs for the length of transport and varying species requirements, to be on the safer end, the loading density would be for 12 hours. [33]

Goal 4: Design shall be safe for human users and the fish ***Objectives*** ***i) Electronic Components:*** Any electronic components incorporated into the design must abide by established aquarium safety standards to mitigate the risk of harm to the stakeholders.			
4.1: Any electronic element incorporated into the design must abide by the safety measures detailed within UL1018. [34]]		As tested by the procedures detailed within UL 1018. [34]	Electronic components of the design must abide by preexisting standards for aquarium elements, to mitigate the risk of electrocution.
Goal 5: The design shall be able to withstand jostling and forces expected of transportation ***Objectives*** ***i) Typical Transportation:*** *Should be able to withstand typical forces and jostling intrinsic to transportation by truck without damage to the design and its functionality.*			
5.1: Must not demonstrate any damage after undergoing emulation of forces and movement typical of transportation		Conduct the drop test, leakproofness test, stacking test, and tear test and observe potential damage as detailed within ISO 16495:2022 [35]. Conduct the rolling test as detailed within ISO 2876:1985 [36].	Furthermore, higher durability ensures a design may be reused, improving the sustainability of a design. Testing methods used for containers intended to hold dangerous materials (ISO 16495:2022) are suggested here as they ensure a high standard for avoiding leaks. Leakage during the transportation process may lead to the suffocation, injury, or death of the fish.
Goal 6: The design must have minimal impact on the environment ***Objectives*** ***i) Dematerialization: s****hould reduce the amount of materials being used in the manufacturing process*			

ii) Disassembly (if applicable): *should be capable of disassembly into its parts following manufacturing and towards end-of-life* ***iii) Carbon Footprint:*** *examining the ecological footprint of differing materials in the design to choose a material that ensures that the lowest footprint produced*			
6.1: Volume must not exceed **2 gallons (0.00757082 m^3)** to transport at maximum 1.5-3 kg fish to save materials	The volume of materials utilized in creating the design must be minimized *The lower the volume of materials used the better*	Measure the volume of the material used on the basis of shape, in **m^3**	The SPSD criteria with regard to optimizing environmental impact [61] outlines this recommendation in the "Raw Materials Stage" of the product design process. Average containers of 2 gallons can be used to hold 1.5-3 kg of fish for up to 12 hours [33], [37], so it has been used as a benchmark for the maximum possible volume used.
6.2: Shall have a **minimum eDiM/assembly time ratio of 0.70**, with the eDiM measured in seconds	*The higher the ratio of (eDiM/assembly time) the better*	The time (s) taken to complete the tasks outlined in [38] for assembly, and then again for disassembly, known as the eDiM (ease of disassembly metric) of a newly produced product to avoid erosion factors [39]. *ratio = eDiM/assembly time*	ISO/TR 14062:2002 [40] highlights how facilitating disassembly is key in being able to reuse product parts to minimize waste and encourage recycling, as well as extend product life. SPSD criteria also highlight "recovery of components for reuse", the disassembly ratio calculated to determine whether the product can be efficiently disassembled. eDiM is a standard tool to compare the disassembly of different designs; however, a baseline can be established based on average assembly vs. disassembly times of products used currently in fish transport [39].
6.3: Must not exceed **5.5 kg of CO_2 emitted per kg** of material used in production,	*The lower the quantity of $kgCO_2e/kg$ for a material produced*	Take all major materials (constituting >10% of production) and	The SPSD criteria highlight ecological footprint as an effective evaluation of sustainability in the choosing of materials for

measured in $kgCO_2e/kg$.	*the better*.	determine the mass of each separately in kg. Convert mass to $kgCO_2e$ using [41] or a similar database depending on the material.	production. Since tanks and containers used in transport incorporate plastic, glass or acrylic PMMA, the corresponding emissions of 1.58 $kgCO_2e/kg$ [42], 1.44 $kgCO_2e/kg$ [43] and 5.5 $kgCO_2e/kg$ can be taken into consideration to determine a limit [44].
Goal 7: The design must facilitate an accessible and pleasant user experience ***Objectives*** ***i) Forces Required for Use:*** *The device should be accessible for the widest range of people and physical capabilities* ***ii) Learning Curve:*** *The time it takes for the user to first set up the design such that it is ready for transport should be minimized* ***iii) Set-up and Take-down Time:*** *The time it takes for the user to set up and take down the design after initially learning its operation should be minimized*			
7.1: The use, transport, repair, and set-up of the design must not require forces or weight exceeding the force capable of the lowest fifth percentile as detailed for various motions within ISO/TR 22411. [45]	The less force required to operate, and lift the design the better	As measured by the use of spring scales to test the force required to complete motions necessary to set-up, use, or take down the device.	Increasing the accessibility of the design increases the usability as defined within ISO 9241-11. [46] Furthermore, it increases the design's "suitability for the widest range of use" as defined as an integral element of accessibility within ISO 9241-20. [47]
7.2	The less time spent to learn how to use the design for the first time the better[46]	As measured by timing an individual's first time learning how to use the device from first being	ISO 9241-11 highlights the intuitive design of a device to facilitate quick and easy learning of the device's set-up, operation, and take-down improving the design's

		introduced to the design to successful set-up and preparation for transportation.	usability and concomitant user experience. [46]
7.3	The less time required for set-up and taking down the device the better	As measured by timing how long it takes an individual to set up the device to be prepared for transportation.	ISO 9241-11 notes the importance of the time spent completing a task in improving a user's perception of the design's efficiency, improving usability and associated user experience. [46]

9. Reference Designs

There are many pre-existing ways of transporting fish while maintaining water quality or reducing stress, but they all have weaknesses and do not satisfy our requirements, providing us with the viable opportunity to improve pre-existing solutions.

9.1 Dark-Insulated Shipping and Double Plastic Bagging

Figure 4: The plastic bags The Beastiary receives fish in.

Figure 5: Water double bagged and taped to round the corners of the bag to avoid fish getting stuck in the corners.
Source: Adapted from [48]

Double bagging the fish and placing them in a box insulated by Styrofoam or other packing materials is considered “standard practice” for most ornamental fish transport. [9], [13], [48], [49], [50] and is used by The Beastiary, as mentioned in the interview transcript in Appendix E. Pure oxygen is often pumped into the bag before transit, with the Calgary Aquarium Society noting that 75: 25 is the

ideal air-to-water ratio in plastic bags to transport fish [51]. This method, as suggested by the Southern Regional Aquatic Center and PBS Pet Travel, helps keep their environment warmer and darker. [50], [62] This design excels with maintaining lighting (requirement 2.3), oxygen levels (requirement 1.6), and temperature (requirement 1.1) as the styrofoam padding, acts as a good insulator for heat and keeps the fish in the dark. However, they are not environmentally friendly, as single-use plastic bags produce 1.58kg of CO_2e for a mass of 6g, much higher than in requirement 6.3 [52], [53], . Additionally, this method requires extra additives, such as buffers, to keep metrics like hardness and pH in check. Scientifically, this extra room results in the sloshing of water, violating requirements 2.2 and 5.1. From the Materials and Molecules course knowledge, polymers can easily be stretched, melted or poked, making this a potential hazard from requirement [5.1].

9.2 Breather Bags:

Figure 6: Kordon Breathing Bag
Source: Adapted from [54]

The breather bag, such as the one by Kordon products, (Figure 6) is micro-porous, allowing for the transfer of gas molecules through the plastic walls of the bag. Therefore, outside oxygen can dissolve into the water once the bag as the fish consumes it, while the excreted CO_2 can escape. Furthermore, the bags are thick, reducing the likelihood of being punctured and leaking [55]. It addresses requirement [2.2] as Kordon Products claims that the bag can be entirely rotated without disturbing the fish. However, for optimal breathability in the breathing bags, they need to be packaged inside of cardboard boxes or Polystyrene. Their breathability also needs higher temperatures, which may not always be possible depending on surrounding temperatures. Furthermore, each bag needs to be separated from another for the gas exchange to occur, requiring extra space, and violating requirements [3.1]. The bags also fail to carry larger fish with higher oxygen demands [55]. These bags are made from polyethylene, rubber and polyester, where materials like polyester can generate 14.2kg of CO_2e for 1kg of polyester, failing [6.3]. Moreover, it is noted that breather bags typically have a higher mortality rate than typical plastic bags as fish are generally more anxious without air physically in the bag, violating Goals 1 and 2 [9].

9.3 Stressguard:

Figure 7: Stressguard by Seacheam used in fish transport to reduce stress and promote healing [56]
Source: Adapted from [56]

The Stressguard by Seacheam (Figure 7) is a slime coat protection, binding to exposed proteins on the fish's skin, giving it a gel-like protective coating. It acts as a barrier against the surrounding environment, making it less prone to irritations such as disease and ammonia detoxification, overall reducing stress in the fish. This barrier also prevents infections, allowing for the healing of wounds. It primarily addresses requirement 2.4, and a research study on a similar product, StressCoat, found a 50% reduction in erratic swimming during transport on arrival of the fish [57]. Also, due to its simple use with addition straight into the water, it excels in requirements associated with user-friendliness, Goal 7. However, this design does not address water quality and will, therefore, fail associated requirements, Goal 1, without the addition of secondary buffers or different containers.

9.4 Clove Oil:

Figure 8: Bottle of clove oil used in fish transport to induce varying levels of anesthesia and sedation [58]
Source: Adapted from [59]

Clove oil is often used and widely recognized as a natural sedative for ornamental fish transport, especially as it is seen as a natural alternative to anesthetics in use. It is directly added to the water and helps the activity of the fish, reducing stress, oxygen demand, and metabolism (consequently ammonia levels). [58] A major advantage is regarding safety, since the dosage can be altered within a large range without harming the fish and satisfies [2.4 and 2.5] overall fish stress levels and movement. However, it does not ensure water quality control and without other additives, would fail all other requirements of Goal 1 such as temperature and pH levels. Its use is also under consideration due to potential environmental impacts from released tank water, as the primary ingredient, eugenol, has been considered an "equivocal carcinogen" in humans [60]. Additionally, the dosage is relatively strict, requiring careful monitoring to prevent over or under-dosage. This makes it difficult to learn and use, making it less favourable under [7.2 and 7.3].

Overall Summary:

Figure 9 summarizes the pros and cons of these reference designs concerning our high-level requirements and tests.

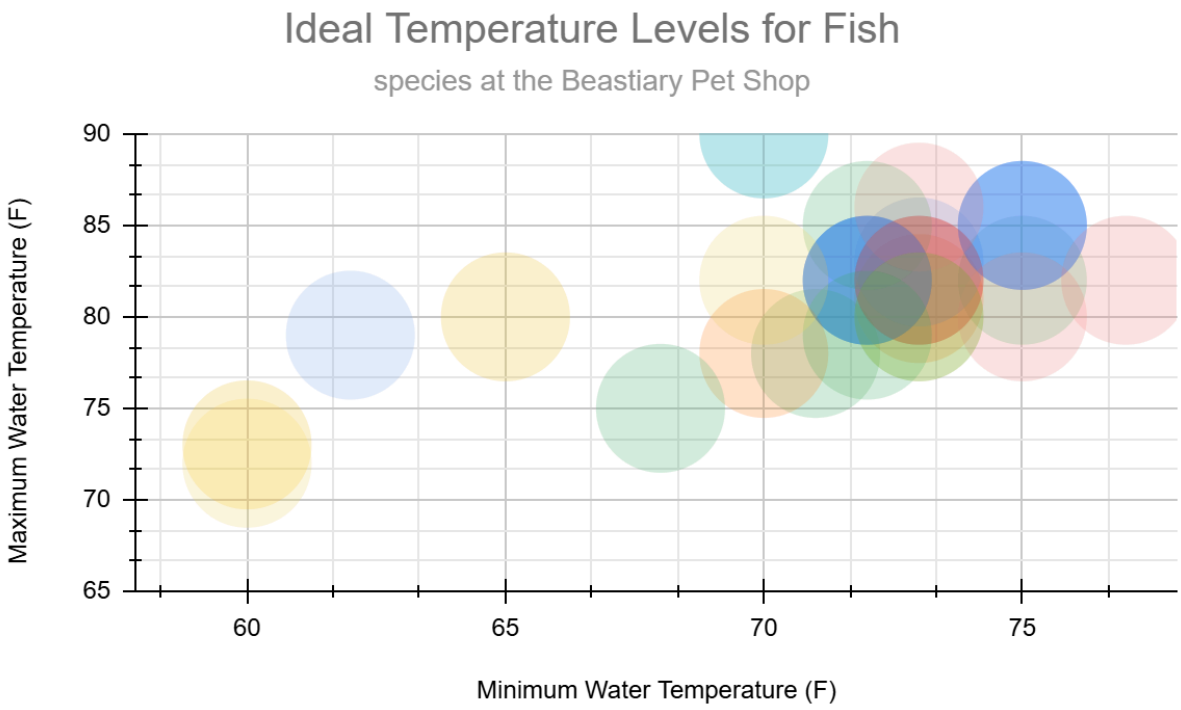
	1.1- 1.4 + 1.6	1.5	2.2 + 5.1	3.1	6.1	6.3
10.1: Plastic Bags	insulation for lighting, temperature and oxygen. Not necessarily for change in pH or water hardness	No chemicals are in direct contact to the fish	Air in bag allows for water sloshing. Bag can be punctured.	Can hold a lot of fish based on bag size.	Designed for portability. Depending on bag size, volume can be less than 2 gallons	Not biodegradable and 1.58kg of CO_2 emissions to produce a 6g bag.
10.2: Breather Bags	Thicker than plastic bags and allow for insulation. Cannot monitor water hardness and pH levels	No chemicals involved	Minimal to no sloshing, and prevents puncture	Because of pressure and breathability, cannot have as many fish. Breather bags need to be separated.	Depends on bag size	Include polyester and polyethlyene, which violate requirements
10.3 Stressguard	The product does not do anything to maintain water quality. Does reduce ammonia toxicity	Chemical coating on the fish	Does not do anything to prevent it, but it prevents stress caused by sloshing	Can apply coating on many fish. Does not directly relate to transportation mechanism		packaged in a reusable plastic bottle.
10.4 Clove Oil	Does not maintain water quality	Chemical coating present; but it's a natural chemical (in form of oil)	Does not prevent it, but prevents stress in fish caused by sloshing	Can apply coating to many fish. Does not directly relate to transportation mechanism		in a reusable plastic bottle. Clove oil is also natural

Figure 9: A table summarizing the pros (green checkmark), and cons (red X) along with factors that were not the purpose of the design (grey O).

10. Conclusion

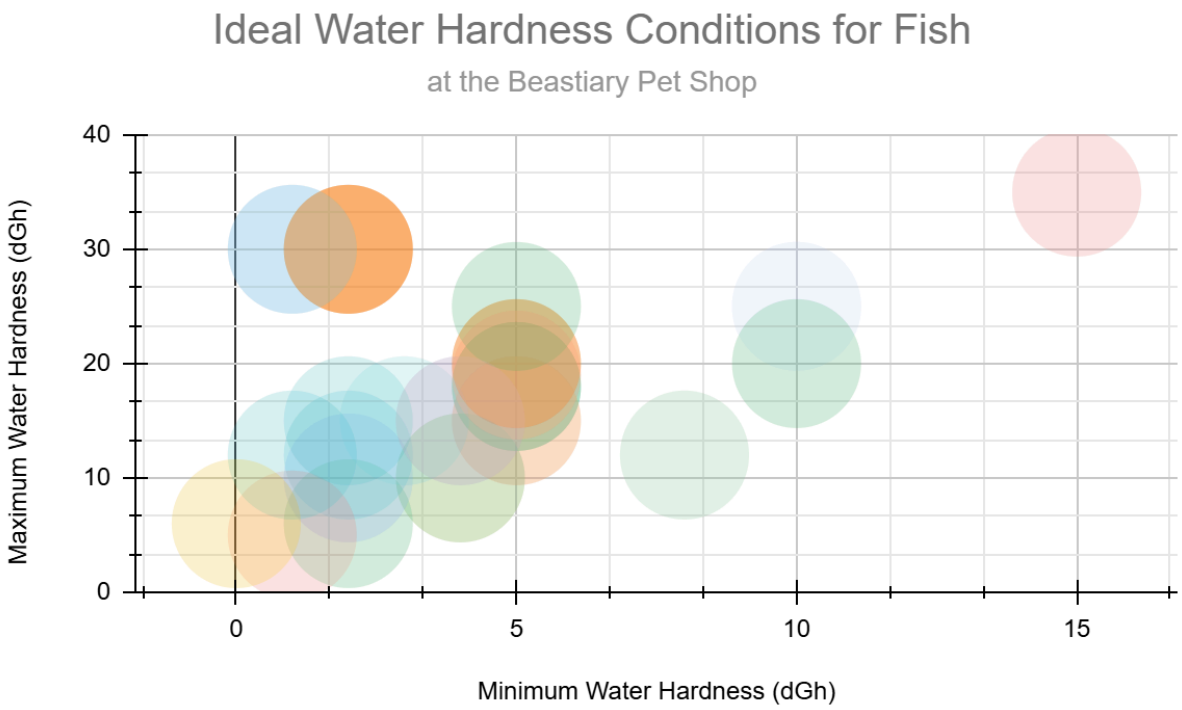
Reducing Ich flare-ups as fish are transported to the Beastiary Pet Shop is significant because it can lead to health problems and death if not prevented or treated adequately. Additionally, Ich is contagious and can rapidly spread to larger fish populations. Existing reference designs do not fulfill all the requirements for a sustainable solution, which prompts the need to find a design that can address the need to lower stress in fish during transportation. The ideal device prioritizes the health and well-being of the fish, as well as the safety of the people operating it. Addressing this opportunity changes the risky and tumultuous, but necessary process of transporting fish into a secure process for both fish and fish owners.

Appendix A: Ideal Temperature Levels for Fish Species



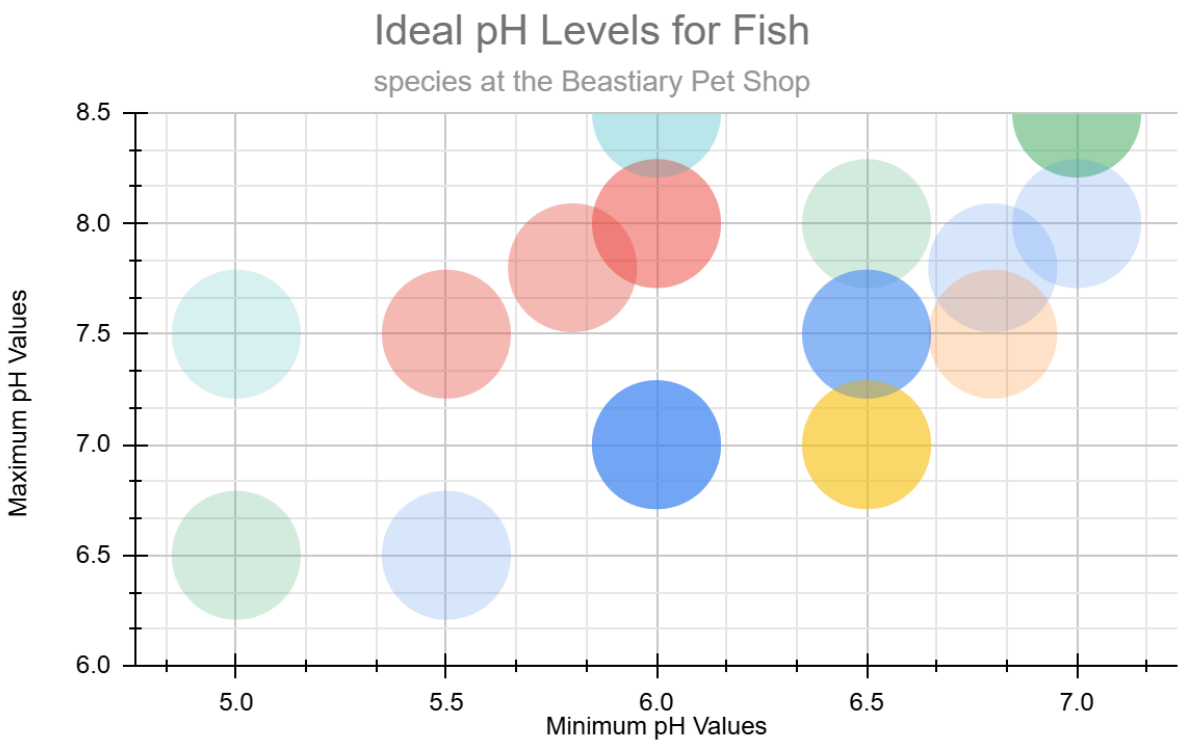
Each circle represents a different fish type sold at the Beastiary Pet Shop and the corresponding required temperature range. It is noted that the most overlap occurs at 71.5°F, which can be rounded to 72°F because most of the collected data from the website had temperature ranges starting from 72°F. The corresponding maximum is at 82°F.

Appendix B: Ideal Water Hardness Levels for Fish Species



Each circle represents a different fish type sold at the Beastiary Pet Shop and the corresponding required water hardness range. It is noted that the most number of overlaps occur at 5dGH which has a corresponding maximum water hardness value of 20dGH.

Appendix C: Ideal pH Levels for Fish Species



Each circle represents a different fish type sold at the Beastiary Pet Shop and the corresponding required pH range. It is noted that the most number of overlaps occur throughout 6 and have a maximum near 7 8 or 8.0, making it our standard.

Appendix D: Loading Density of Fish

Fish Size	Loading Density (Pure Oxygen) g/L	Loading Density (Diffused Oxygen) g/L
¼ Inch	50	30
1 inch	100	120
2 inch	105	120
3 inch	105	240
Larger Fish	180	360

From the University of Arizona for transporting fish over 12 hours for common ornamental fish sizes. [33] Pure oxygen is the injection of oxygen in an air-tight bag before transport, similar to reference design 9.1. Diffused oxygen is the exchange of oxygen during transport, similar to reference design 9.2.

Appendix E: Interview Transcripts

Phone call with Alex - Feb 14 2025:

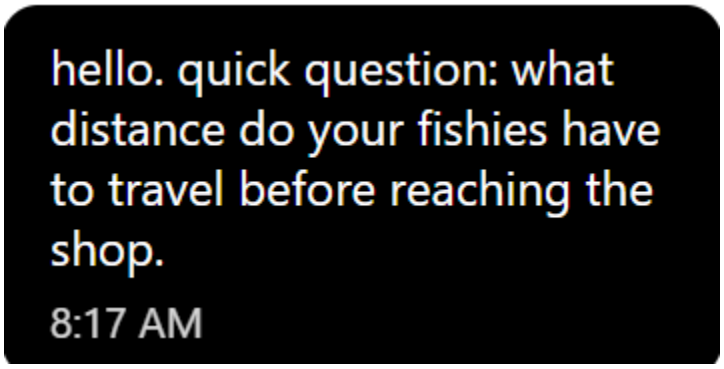
Notes

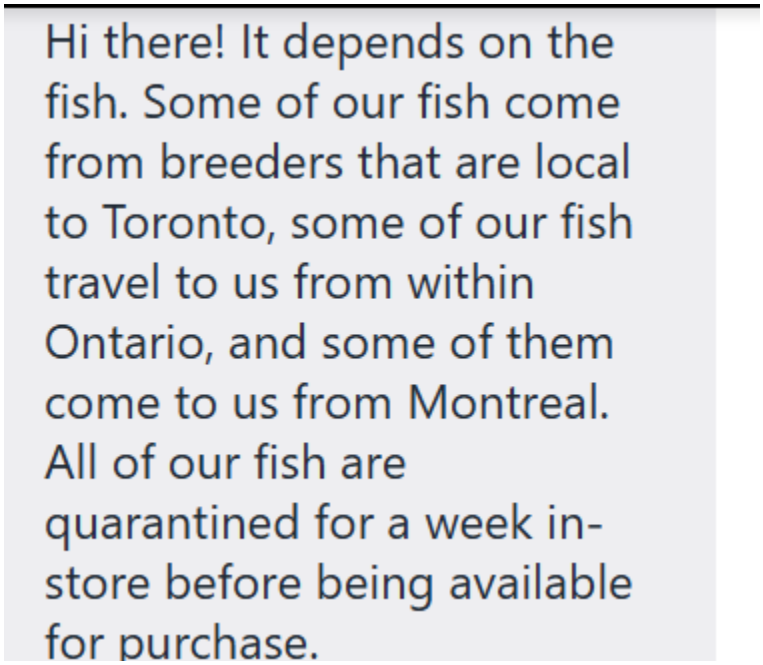
Where are fish transported from?

Majority from just outside of Toronto, basically none from Northern Ontario because there are no international airports because most fish are flown in from Asia. Also source fish from MSR Imporium Canada in Montreal, farthest major distributor for the pet shop.

Use air compression used to store enough oxygen for the fish to last them one to two days.

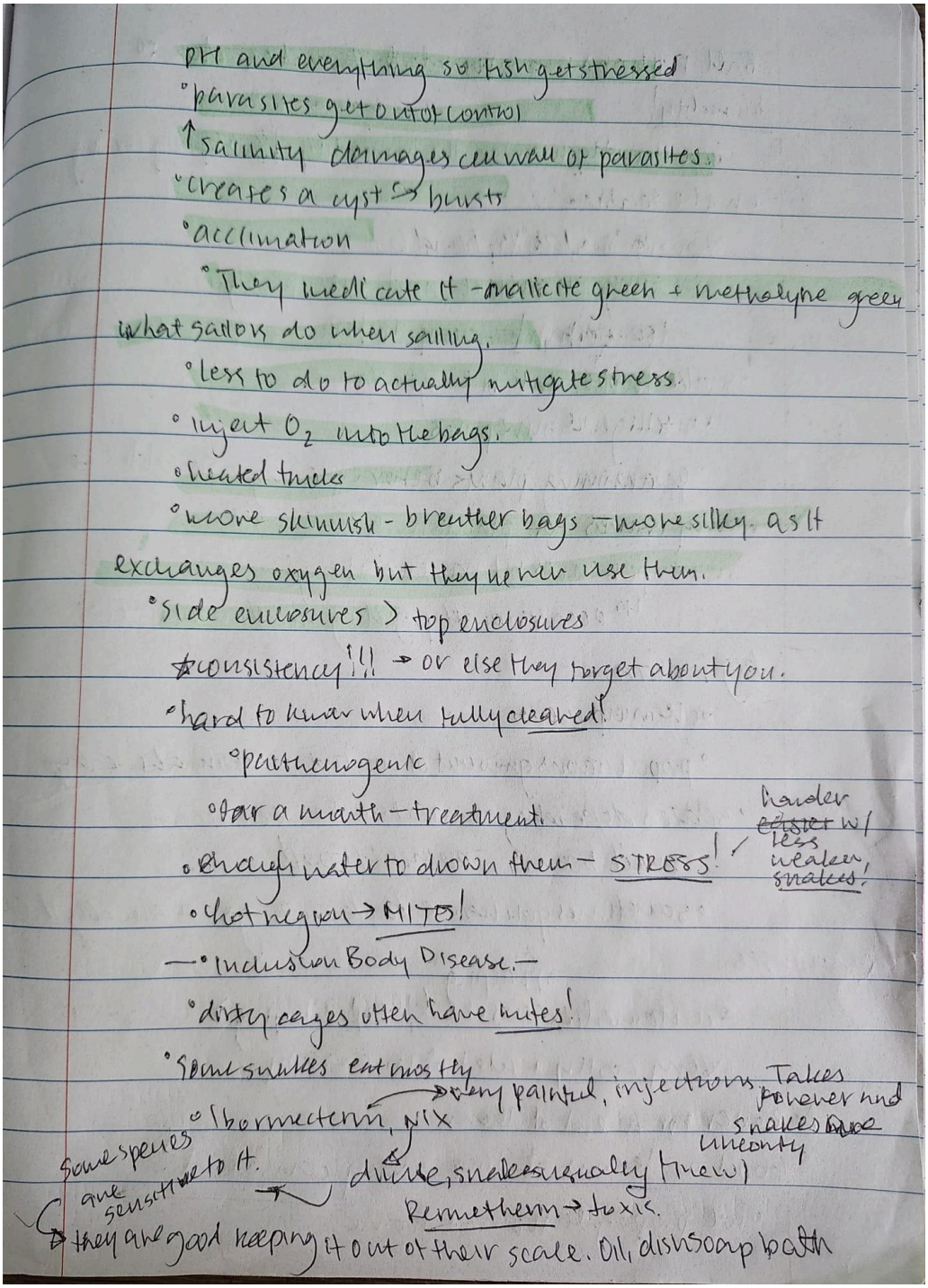
Conversation over website's live chat:

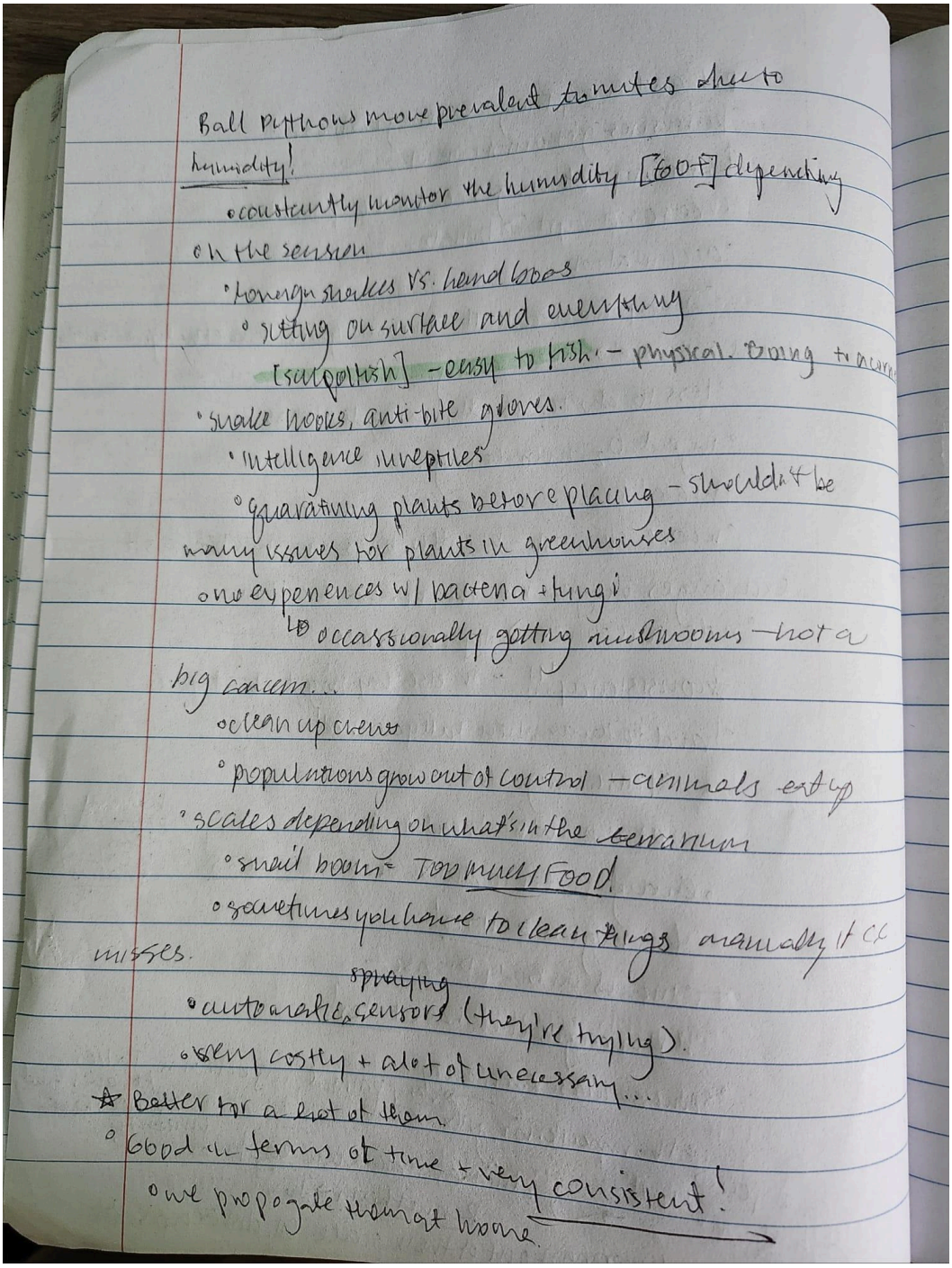




Second In-Person Meeting with Beastiary Pet Shop - Feb 10 2025

Highlighted lines are specific to the opportunity of fish transportation





First In-Person Meeting with the Beastiary Pet Shop (relevant to opportunity)

Maintenance- fish tanks, feeding, cleaning

Animals? ~100 reptiles, several hundred fish

Customers are the most difficult part of working; difficult to listen (10 gallon tank but put in a 5 gallon)

Goldfish bowl - originally 20 gallon bow

Quarantine room for new fishes before giving to sale (percent have sickness and need to be made healthy)

Critter carrier containers deli cups with holes and lids

Bags to transport

- placed in aquarium in bag to get used to water temp
- Ick disease persistent on them, dormant until high stressed due to dirty water
- Fish to ensure no ick (3 days to 1 week)

Tank leakages, floor wear and tear unexpected

References and Source Extracts

[1] . *CIR920/FA006: Ichthyophthirius multifiliis (White Spot) Infections in Fish*. Available: https://edis.ifas.ufl.edu/publication/FA006

Ichthyophthirius multifiliis is a relatively large, single-celled ciliated protozoan that causes "Ich" or "white spot disease." This disease is a major problem to freshwater aquarists and commercial fish producers worldwide. All species of freshwater fish are considered susceptible, and the parasite has been found in all areas of the world in both cultured and wild fish. Although large, these parasites do require a microscope to confirm them as a cause of the characteristic white spots that are often seen on the skin and fins of infected fish (Figure 1). The disease is highly contagious and spreads rapidly from one fish to another without the need for additional hosts (direct life cycle). Although outbreaks may occur at any time, they often appear when water temperatures are changing more rapidly. In the spring, when water temperatures are increasing, the Ich life cycle proceeds more quickly in warmer temperatures; conversely, in the fall and winter, decreases in fish immune function will also favor infection. The disease is particularly severe when fish are crowded. While many protozoans reproduce by simple division (one parasite "splits" into two), a single Ich organism can multiply into hundreds of new parasites in one generation, making early detection and treatment of this parasite crucial. The organism is unusual in that it is an obligate parasite, which means that it cannot survive unless live fish are present. Ich is capable of causing massive mortality within a short period of time. An outbreak of Ichis a true emergency situation and requires immediate treatment; if left untreated, this disease may result in 100% mortality.

Although *Ichthyophthirius multifiliis* has a direct life cycle (i.e., it reproduces without a need for another animal species host) (Figure 2), the life cycle is complex and has three distinct life stages: 1) the on-fish, feeding trophont (Figure 3); 2) the environmental, reproducing tomont (Figure 4); and 3) the infective, fish-seeking theront (see Figure 5). The trophont invades and encysts between the thin outer layers (epithelium) of the fish host's skin and gills to feed on those tissues (Figure 6). Because it is covered by the fish's epithelial tissue and mucus, the adult parasite, or trophont stage, is protected from chemical treatment. Once the trophont is mature, it stops feeding, leaves the fish, and becomes a tomont. The tomont quickly secretes a gelatinous-walled outer cyst (Figure 4) that allows it to stick to surfaces in the environment. Some references consider the tomont to be the stage that leaves the fish, becoming a tomocyst after it settles and forms a cyst, but for purposes of EDIS we will use the more traditional life cycle as described in Figure 2. The tomont begins to divide quickly up to 10 times, forming as many as 1024 new "daughter" parasites (tomites) within a single cyst (Figure 4). This can occur in 18 – 24 hours at warmer water temperatures (23°C/74°F). The gelatinous wall of the tomont cyst protects it and the daughter tomites from chemical treatment. The tomites begin to develop and become theronts within the tomont cyst. Following a period of days (warm water temperatures) or weeks (cool water temperatures), the theronts bore out of the tomont cyst and become free-swimming, infective parasites in search of a fish host. These infective theronts must find a live fish to complete the parasite's life cycle. **This free-swimming phase is unprotected and, therefore, highly susceptible to chemicals.** Treatment protocols should be designed to target this theront stage.

[2] (-05-02). *Ich Disease: How to treat white spot*. Available: https://aquacadabra.com/blogs/news/how-to-treat-white-spot.

White spot disease – what is it?

At the outset, it is worth mentioning that this disease looks similar in both marine and freshwater aquariums, but treat ich is quite different. However, it is caused by two other types of *ciliata*. They differ in the structure of the macronucleus. In the case of freshwater, it is a disease caused by a parasite called ***Ichthyophthirius multifiliis*** (Ich). In marine aquariums, we call this parasite as ***Cryptocaryon irritans.***

These dangerous protozoa can infect any fish species in the aquarium.
Good to know: Did you know that Ichthyophthirius multifiliis as a parasite is large enough to be seen with the naked eye? It reaches up to 1.5 mm in length.

[3] Quebec Cichlides. . *Freshwater Ich : Symptoms, Causes and Treatment*. Available: https://quebec-cichlides.com/en/freshwater-ich-symptoms-causes-and-treatment/.

Causes

Ich is a widespread disease. You might have some in your aquarium and not even know it. Lucky for us, our fish have a good immune response to this parasite and you might never see any symptoms appear on your fish.

Stress

Stress is the main factor in ich eruptions. If your fish get stressed, either by their environment, transportation, their tank mates or because they can't remember if they left the stove on, their immune system get weakened and they will become susceptible to an ich infection.

Even though transportation is stressful for the fish, if you acclimate them to a low-stress environment, they shouldn't get infected.

[4] "Treat ich on fish - guide," Available: https://aquaforest.eu/en/knowledge-base/treat-ich-on-fish-understanding-treating-and-preventing-white-spot-disease/.

White spot disease – what is it?

At the outset, it is worth mentioning that this disease looks similar in both marine and freshwater aquariums, but treat ich is quite different. However, it is caused by two other types of *ciliata*. They differ in the structure of the macronucleus. In the case of freshwater, it is a disease caused by a parasite called ***Ichthyophthirius multifiliis*** (Ich). In marine aquariums, we call this parasite as ***Cryptocaryon irritans.***

[5] H. W. Dickerson, "(PDF) Ichthyophthirius multifiliis and Cryptocaryon irritans (Phylum Ciliophora)," Available: https://www.researchgate.net/publication/284902551_Ichthyophthirius_multifiliis_and_Cryptocaryon_irritans_Phylum_Ciliophora.

C. irritans is not fastidious in its host selection (Colorni, 1985). The host range does not appear to be as great as that of *I. multifiliis*, however. Fishes vary in susceptibility to infection. Elasmobranchs are generally resistant (Wilke and Gordin, 1969). Also, there are apparent differences in degrees of susceptibility in marine teleosts.

[6] S. B. University. (february 8). *Why are there so few fish in the Earth's oceans?*. Available: https://phys.org/news/2012-02-fish-earth-oceans.html.

"There are more fish species in freshwater than in saltwater habitats, despite the much greater area and volume of the oceans," he said, noting that freshwater environments occupy only about 2 percent of the Earth's surface. "More remarkably,

[7] . *The Beastiary Pet Shop*. Available: https://thebeastiary.ca/products/dwarf-neon-rainbowfish.

Temperament: Schooling species, peaceful and active. May be timid in too small of a school. Best results in large groups of 10+ members.

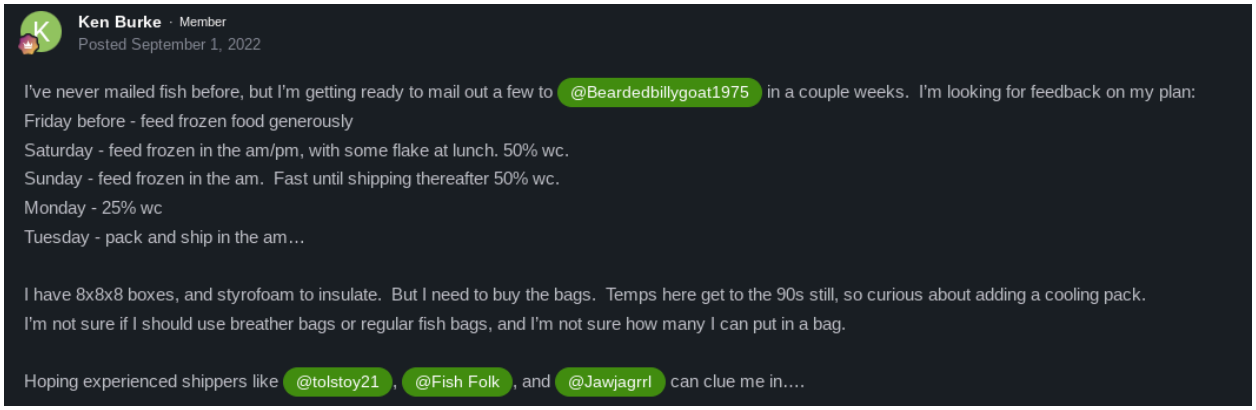
[8] . *How To Move Your Fish Locally Or Long Distance*. Available: https://www.moveeast.com/moving-guide/moving-animals-and-plants/moving-fish/.

Your fish should also be the very first thing to be unpacked once they have reached the final destination. Transportation Options For Your Fish

- One way to transport fish is in plastic bags. Special plastic fish bags can be obtained from pet stores.
- Fill the bag a third of the way using water from the fish tank or aquarium. Use a separate plastic bag for each fish. Do not put more than one fish in each bag.
- For extra protection and to reduce leaking, use a second plastic bag to cover the first bag.
- Using a rubber band, close the two bags securely.
- If the fish will be in bags for more than one hour, place pure oxygen in the bags.
- If you have multiple fish, transporting them in buckets is an easy way to move them.
- Using a 5 gallon bucket is advised.
- Use a new bucket. Do not use a bucket that has had, or may have had chemicals in it.
- Make sure the bucket has a water tight lid.
- Fill the bucket with water from your tank or aquarium.
- A container could also be used to transport your fish. Make sure it is strong, durable and has a lid.
- Use water from the tank or aquarium and ensure the lid is secure.
- Using a container may be a good way of transporting fish with sharp fins.

- For about 5 days prior to moving, change the water – switch 20% of the water in the aquarium. This will provide clean water in the tank.
- 1 – 2 days prior to moving and transporting do not feed your fish. Fish can survive up to about 7 days without food. This will lessen the chances of the water getting too messy.
- Your fish should be the very last thing to be packed. They should not be waiting while everything else gets packed or loaded. They need to be in transit for as little time as possible.

[9] Ken Burke (-09-02). *Shipping fish*. Available: https://forum.aquariumcoop.com/topic/26580-shipping-fish/.

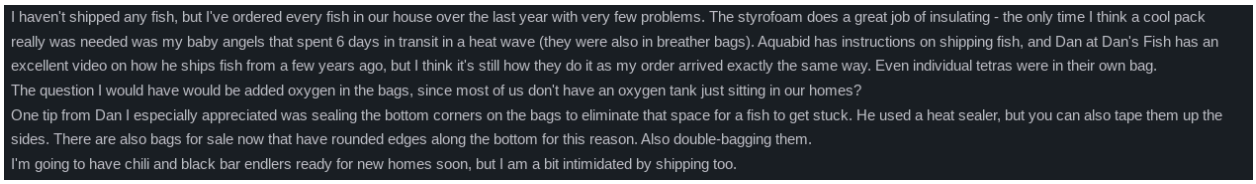


Ken Burke · Member
Posted September 1, 2022

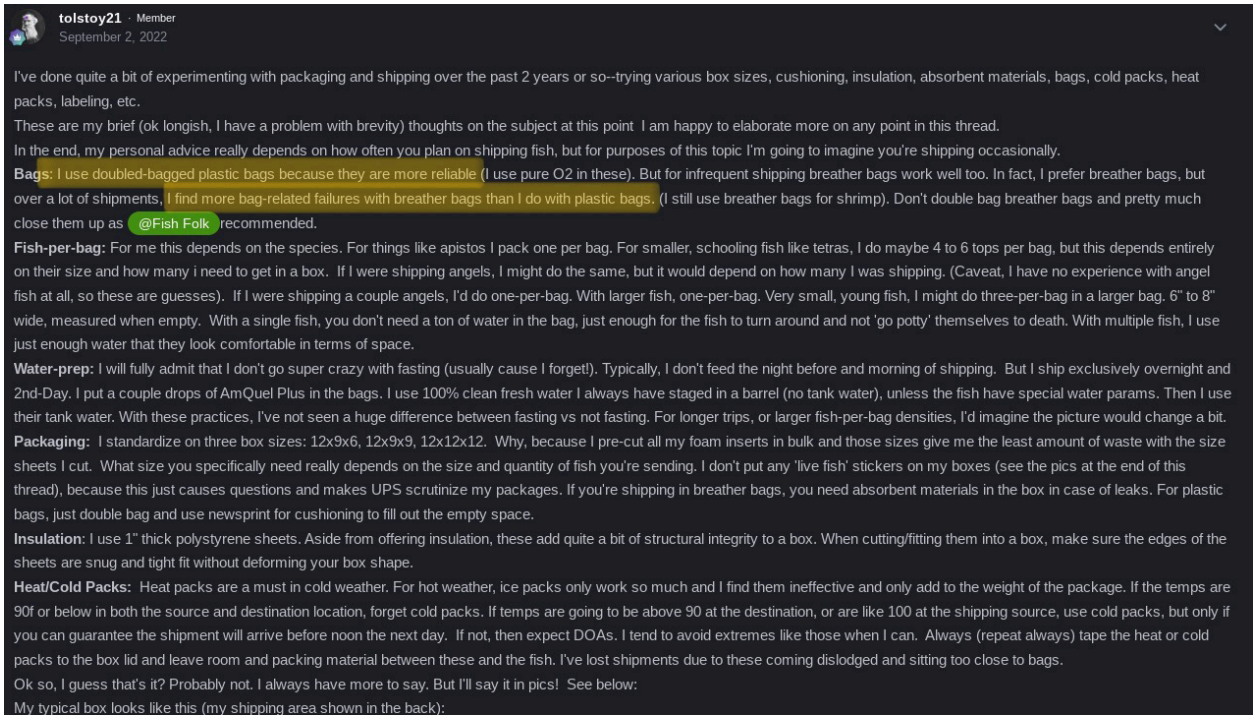
I've never mailed fish before, but I'm getting ready to mail out a few to @Beardedbillygoat1975 in a couple weeks. I'm looking for feedback on my plan:
Friday before - feed frozen food generously
Saturday - feed frozen in the am/pm, with some flake at lunch. 50% wc.
Sunday - feed frozen in the am. Fast until shipping thereafter 50% wc.
Monday - 25% wc
Tuesday - pack and ship in the am…

I have 8x8x8 boxes, and styrofoam to insulate. But I need to buy the bags. Temps here get to the 90s still, so curious about adding a cooling pack.
I'm not sure if I should use breather bags or regular fish bags, and I'm not sure how many I can put in a bag.

Hoping experienced shippers like @tolstoy21, @Fish Folk, and @Jawjagrrl can clue me in….



I haven't shipped any fish, but I've ordered every fish in our house over the last year with very few problems. The styrofoam does a great job of insulating - the only time I think a cool pack really was needed was my baby angels that spent 6 days in transit in a heat wave (they were also in breather bags). Aquabid has instructions on shipping fish, and Dan at Dan's Fish has an excellent video on how he ships fish from a few years ago, but I think it's still how they do it as my order arrived exactly the same way. Even individual tetras were in their own bag.
The question I would have would be added oxygen in the bags, since most of us don't have an oxygen tank just sitting in our homes?
One tip from Dan I especially appreciated was sealing the bottom corners on the bags to eliminate that space for a fish to get stuck. He used a heat sealer, but you can also tape them up the sides. There are also bags for sale now that have rounded edges along the bottom for this reason. Also double-bagging them.
I'm going to have chili and black bar endlers ready for new homes soon, but I am a bit intimidated by shipping too.



tolstoy21 · Member
September 2, 2022

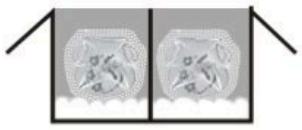
I've done quite a bit of experimenting with packaging and shipping over the past 2 years or so--trying various box sizes, cushioning, insulation, absorbent materials, bags, cold packs, heat packs, labeling, etc.
These are my brief (ok longish, I have a problem with brevity) thoughts on the subject at this point I am happy to elaborate more on any point in this thread.
In the end, my personal advice really depends on how often you plan on shipping fish, but for purposes of this topic I'm going to imagine you're shipping occasionally.
Bags: I use doubled-bagged plastic bags because they are more reliable (I use pure O2 in these). But for infrequent shipping breather bags work well too. In fact, I prefer breather bags, but over a lot of shipments, I find more bag-related failures with breather bags than I do with plastic bags. (I still use breather bags for shrimp). Don't double bag breather bags and pretty much close them up as @Fish Folk recommended.
Fish-per-bag: For me this depends on the species. For things like apistos I pack one per bag. For smaller, schooling fish like tetras, I do maybe 4 to 6 tops per bag, but this depends entirely on their size and how many i need to get in a box. If I were shipping angels, I might do the same, but it would depend on how many I was shipping. (Caveat, I have no experience with angel fish at all, so these are guesses). If I were shipping a couple angels, I'd do one-per-bag. With larger fish, one-per-bag. Very small, young fish, I might do three-per-bag in a larger bag. 6" to 8" wide, measured when empty. With a single fish, you don't need a ton of water in the bag, just enough for the fish to turn around and not 'go potty' themselves to death. With multiple fish, I use just enough water that they look comfortable in terms of space.
Water-prep: I will fully admit that I don't go super crazy with fasting (usually cause I forget!). Typically, I don't feed the night before and morning of shipping. But I ship exclusively overnight and 2nd-Day. I put a couple drops of AmQuel Plus in the bags. I use 100% clean fresh water I always have staged in a barrel (no tank water), unless the fish have special water params. Then I use their tank water. With these practices, I've not seen a huge difference between fasting vs not fasting. For longer trips, or larger fish-per-bag densities, I'd imagine the picture would change a bit.
Packaging: I standardize on three box sizes: 12x9x6, 12x9x9, 12x12x12. Why, because I pre-cut all my foam inserts in bulk and those sizes give me the least amount of waste with the size sheets I cut. What size you specifically need really depends on the size and quantity of fish you're sending. I don't put any 'live fish' stickers on my boxes (see the pics at the end of this thread), because this just causes questions and makes UPS scrutinize my packages. If you're shipping in breather bags, you need absorbent materials in the box in case of leaks. For plastic bags, just double bag and use newsprint for cushioning to fill out the empty space.
Insulation: I use 1" thick polystyrene sheets. Aside from offering insulation, these add quite a bit of structural integrity to a box. When cutting/fitting them into a box, make sure the edges of the sheets are snug and tight fit without deforming your box shape.
Heat/Cold Packs: Heat packs are a must in cold weather. For hot weather, ice packs only work so much and I find them ineffective and only add to the weight of the package. If the temps are 90f or below in both the source and destination location, forget cold packs. If temps are going to be above 90 at the destination, or are like 100 at the shipping source, use cold packs, but only if you can guarantee the shipment will arrive before noon the next day. If not, then expect DOAs. I tend to avoid extremes like those when I can. Always (repeat always) tape the heat or cold packs to the box lid and leave room and packing material between these and the fish. I've lost shipments due to these coming dislodged and sitting too close to bags.
Ok so, I guess that's it? Probably not. I always have more to say. But I'll say it in pics! See below:
My typical box looks like this (my shipping area shown in the back):

[10] B. Blake. (-01-01). *Recommended Freshwater Aquarium Water Parameters: Part 2*. Available: https://www.boodleshireaquatics.com/post/recommended-freshwater-aquarium-water-parameters-part-2.

Ideally, free and total ammonia (ammonia/NH_3, ammonium/NH_4) should be at 0 ppm (parts per million). A spike may elevate ammonia to 0.25, 0.5 or even 1 ppm. Ammonia can be toxic to fish and inverts at any level, but should definitely be considered an emergency above 0.5 ppm.

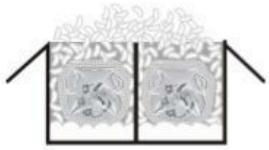
[11] "How to Pack and Ship Breakables | The UPS Store Canada," Available: https://www.theupsstore.ca/how-to-ship-breakables/.

Ensuring the bottom of the box is completely sturdy is important when shipping fragile items. Take a previously unused box and tape down the centre and end seams with rugged packing tape. Open up the box, put the corrugated cardboard dividers in place first and then add a 2-inch (5.08 cms) layer of packing peanuts to the bottom before placing the items in the box.



Use Loose Fill

Once the items are firmly in place, fill the box with packing peanuts to ensure there is as little movement of the items throughout the shipping process as possible. Overfill the box by about 5 to 10 per cent with those packing peanuts as they will settle and fill up any empty space within the box during the trip.

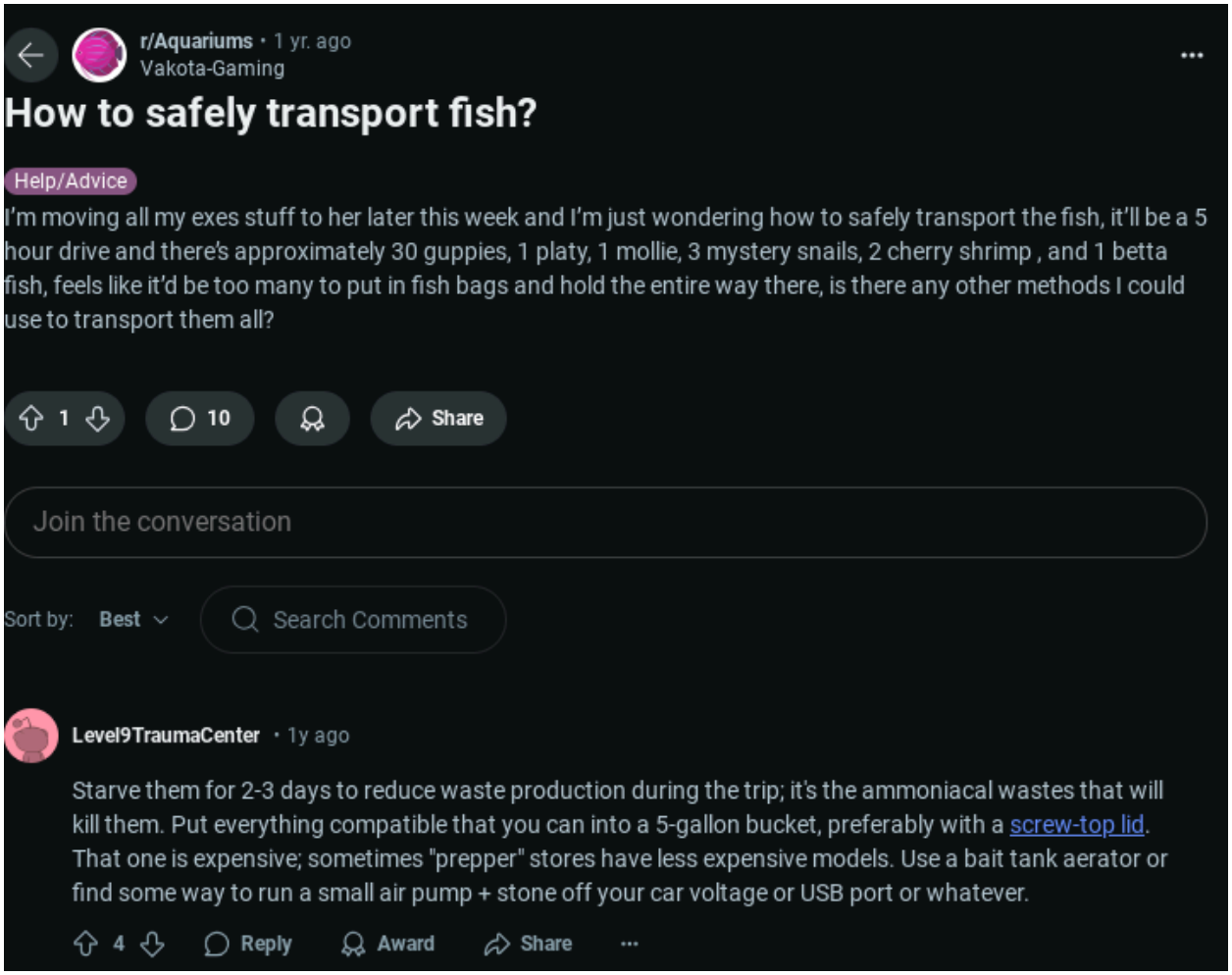


Heavier Items Should Fly Solo

For fragile items that are over 5 pounds (2.3 kgs) but less than 10 pounds (4.5 kgs), they should be boxed individually. As with smaller items, each should be first wrapped in at least three layers of bubble wrap before being placed in the box.

If the fragile item is more than 10 pounds, it should also be boxed individually and then double boxed. This means placing the bubble-wrapped item in a smaller box surrounded by packing peanuts and then placing that box into a bigger one that will also be filled with packing peanuts for an extra level of protection.

[12] Vakota-Gaming, "How to safely transport fish?" 2024. Available: https://www.reddit.com/r/Aquariums/comments/17tqvym/how_to_safely_transport_fish/?rdt=58247.



[13] Yonatan24workshop, "Pro tip: If you need to carry fish in a backpack, put the bag in a PVC pipe, over reusable bags as supportive cushions." 2021. Available: https://www.reddit.com/r/Aquariums/comments/m690vk/pro_tip_if_you_need_to_carry_fish_in_a_backpack/.

[14] "Transport Packaging - Temperature controlled transport packages for parcel shipping - Part 2: General specifications of testing ISO 22982-2:2021," Available: https://subscriptions-techstreet-com.myaccess.library.utoronto.ca/products/894213.

4.3.3 Testing insulation performance of a package

4.3.3.1 General

This test is to measure insulation performance of temperature-controlled packages based on existing or predicted parcel shipping temperature profiles.

4.3.3.2 Insulation performance test conditions

The conditions for insulation performance tests are as follows:

a) test in a chamber that is not influenced by direct sunlight, heat sources and can maintain and adjust temperature with less than or equal to ±1 °C during the measurement period;

b) preheat or pre-cool the package until the inside temperature is stabilized;

c) measure the temperature every 10 min;

d) mark the measurement temperature as the mean value of measurement points inside the chamber (package outside) and the package inside respectively; and

e) equalize the inside temperature of the package.

4.3.3.3 Placement of sensors for temperature measurement

For common practice, at least 3 sensors are needed for the accurate temperature measurement. Sensors shall be located upper middle, centre, and lower middle of the package, respectively. If a limited number of sensors is available, it is recommended to place them in the following order: lower middle, upper middle and centre of package. The sensor shall not be directly attached the product or cooling agent such as PCM, dry ice, etc. The inside and outside temperature of the package should be continuously recorded and reported.

4.3.3.4 Placement of cooling agent in a package

Cooling agents such as ice packs and PCM may be used to maintain optimum temperature of the package inside. It is recommended to place a cooling agent on the upper middle and 4 sidewalls in a

[15] H. Canada. (-08-06). *Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Hardness*. Available: https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-guideline-technical-document-hardness.html.

strontium, iron, barium and manganese ions also contribute. [1] Hardness ca
be measured by the reaction of polyvalent metallic ions in a water sample wit
a chelating agent such as ethylenediaminetetra-acetic acid (EDTA) and is
commonly expressed as an equivalent concentration of calcium carbonate. [1]
[2] Hardness can also be estimated by determining the concentrations of the
individual components of hardness and expressing their sum in terms of an
equivalent quantity of calcium carbonate. The degree of hardness of drinking

[16] E. Heath, "How to Test and Balance Pool pH Levels," 2022. Available: https://www.familyhandyman.com/article/how-to-test-and-balance-pool-ph-levels/.

To conduct your own water testing, you'll need at minimum a container of chemical test strips. Dip a strip in the pool water, then measure the readout against the corresponding colors printed on the container. These show acceptable ranges for pH, bromine, chlorine and other measures.

The healthy pH range for a swimming pool is between 7.4 and 7.8. Most home kits won't give you a numerical reading, but the "good" color range on the test control indicates an optimal pH.

[17] *Freshwater Aquarium Water Quality: The Nitrogen Cycle & Optimal Water Chemistry*. Available: https://www.aqueon.com/articles/freshwater-aquarium-water-quality.

pH – the measure of whether water is acidic (pH 1 to 7.0) or basic (pH 7.1 to 14). 7.0 is considered neutral. Most freshwater aquarium tropical fish do best at a pH of 6.8 to 7.8, although certain fish may require higher or lower levels. The pH of an aquarium tends to drop over time due to the breakdown of organic material, and the best way to prevent this is through regular partial water changes.

[18] *Why is testing aquarium water important?*. Available: https://www.aqueon.com/articles/why-is-testing-aquarium-water-important.

You can easily watch your ammonia levels with Aqueon Ammonia Test Strips. Dip a strip into the aquarium water, remove the strip, wait about two minutes, then compare the color-coded results to the provided chart. This will let you quickly determine if your ammonia levels are safe.

[19] Services, Florida Department of Agriculture and Consumer and wilton simpson. . *Aquarium Water Quality: Nitrogen Cycle / Aquarium Fish / Recreation and Leisure / Consumer Resources / Home - Florida Department of Agriculture & Consumer Services*. Available: https://www.fdacs.gov/Consumer-Resources/Recreation-and-Leisure/Aquarium-Fish/Aquarium-Water-Quality-Nitrogen-Cycle.

Ammonia is formed from the metabolism of protein and is the major waste product of fish. The majority of ammonia from fish is excreted through the gills, with relatively little being lost through urine and feces. Ammonia is also formed as uneaten feed or other organic matter in an aquarium decomposes. High concentrations of ammonia in the water make it difficult for fish to eliminate ammonia from their bodies. This buildup of ammonia can cause stress, gill and internal organ damage, and eventually death.

Total ammonia is comprised of two components: un-ionized ammonia (NH3) and ionized ammonia (NH4+). Un-ionized ammonia is extremely toxic to fish whereas ionized ammonia is not. The proportion of un-ionized to ionized ammonia shifts in relation to pH and water temperature. As pH or temperature increases, more of the ammonia shifts to the un-ionized, toxic form. Un-ionized ammonia begins causing gill damage at approximately 0.05 mg/L and death at approximately 2.0 mg/L. Keep in mind that most test kits measure total ammonia and not un-ionized ammonia but may make no reference to the difference. A table is used to calculate the portion of un-ionized ammonia from total ammonia.

[20] Mr John Batt *et al*, "CCAC guidelines on: the care and use of fish in research, teaching and testing, 2005," 2005. Available: https://ccac.ca/Documents/Standards/Guidelines/Fish.pdf.

6. Collection of Body Fluids

Guideline 101:

Sedation or anesthesia should be used to restrain fish for collection or cannulation purposes. It is important to realize that both restraint and anesthesia may alter physiological parameters such as serum glucose and various hormone levels.

p. 59

In laboratory or applicable field situations, fish must receive careful attention and monitoring following surgery.

Although recovering fish may appear to be normal, there may be prolonged metabolic effects following the stress of anaesthesia and surgery. In situations where monitoring is not possible, pilot scale evaluations of procedures should be considered. Where possible, fish should be allowed to recover from anesthesia until able to resume normal behavior. As anesthesia itself causes prolonged stress, careful procedures for recovery are vital, for example, a quiet, well aerated, possibly darkened tank will facilitate recovery.

Guideline 85:

During prolonged surgery, water quality should be maintained at a high level, with minimal bacterial and organic burden. Water for anesthesia should be from the same source as the tank water to minimize shock caused by differences in temperature, pH, electrolytes, etc.

Section 3.2 Water quality during surgery, p. 53

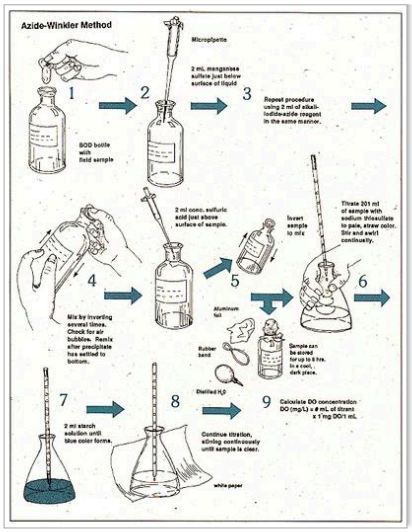
[21] "Anaesthesia of Finfish," Available: https://ccac.ca/Documents/Education/DFO/2_Anaesthesia_of_Finfish.pdf

- Have trainees make 50 mls of a 50 mg/ml stock solution of TMS™. 2.5 grams of TMS™ are mixed with distilled water to make a total volume of 50 mls.
- Prepare a 100 mg/ml stock solution of sodium bicarbonate if this exercise is being preformed in fresh water. (5 grams of NaHCO3 are mixed with distilled water to make a total volume of 50 mls).
- Transfer the stock solution of TMS™ to an amber Nalgene bottle.

[22] M. Z. Bruckner. . *The Wrinkler Method - Measuring Dissolved Oxygen*. Available: https://serc.carleton.edu/microbelife/research_methods/environ_sampling/oxygen.html.

How to– Sample Collection, Preparation, Analytical Protocols, and Concerns

Dissolved oxygen should be measured as quickly and carefully as possible. Ideally, samples should be measured in the field immediately after collection. The following protocol is adapted from Washington State University's How to Measure Dissolved Oxygen (more info) website.



▶ Show credits

Reagent List:

- 2ml Manganese sulfate
- 2ml alkali-iodide-azide
- 2ml concentrated sulfuric acid
- 2ml starch solution
- Sodium thiosulfate

These reagents are available in dissolved oxygen field kits, such as those made by the Hach Company. Please use caution when using these reagents, as they can be hazardous to one's health.

Procedure:

1. Carefully fill a 300-mL glass Biological Oxygen Demand (BOD) stoppered bottle brim-full with sample water.
2. Immediately add 2mL of manganese sulfate to the collection bottle by inserting the calibrated pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via the pipette.
3. Add 2 mL of alkali-iodide-azide reagent in the same manner.
4. Stopper the bottle with care to be sure no air is introduced. Mix the sample by inverting several times. Check for air bubbles; discard the sample and start over if any are seen. If oxygen is present, a brownish-orange cloud of precipitate or floc will appear. When this floc has settle to the bottom, mix the sample by turning it upside down several times and let it settle again.
5. Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully stopper and invert several times to dissolve the floc. At this point, the sample is "fixed" and can be stored for up to 8 hours if kept in a cool, dark place. As an added precaution, squirt distilled water along the stopper, and cap the bottle with aluminum foil and a rubber band during the storage period.
6. In a glass flask, titrate 201 mL of the sample with sodium thiosulfate to a pale straw color. Titrate by slowly dropping titrant solution from a calibrated pipette into the flask and continually stirring or swirling the sample water.
7. Add 2 mL of starch solution so a blue color forms.
8. Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue color. Be especially careful that each drop is fully mixed into the sample before adding the next. It is sometimes helpful to hold the flask up to a white sheet of paper to check for absence of the blue color.
9. The concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used. Each mL of sodium thiosulfate added in steps 6 and 8 equals 1 mg/L dissolved oxygen.

[23] R. Berka, "The transport of live fish A review," *FAO,* Available: https://www.fao.org/4/af000e/AF000E00.htm#TOC.

The first hour after loading is a particularly critical time for fish in respect to their oxygen needs. They are excited and require a large amount of oxygen with a short time for adjustment. Significant differences in oxygen demand exist also within fish families. As asserted, for instance, by Uryn (1971), when water temperature increases (4–14°C) during transport, the fry of *Coregonus lavaretus* consume 2.4 times more oxygen than the fry of *C. albula*. Fish size is also important. A large fish consumes less oxygen per unit weight than does a small one. Oxygen levels of water for most warm water fish should be above 5 $mg.1^{-1}$ for normal conditions. This level should prevent oxygen from becoming a major stress factor.

[24] J. Samp. (-11-25). *Understanding Dissolved Oxygen in Aquariums: Why It Matters and How to Measure It*. Available: https://sensorex.com/aquarium-supply-dissolved-oxygen/.

Before you think about adding your fish back into the tank water, **it's important that you check the water temperature, chlorine levels, ammonia levels, and pH balance**. With the right sensors, all of these measurements can be obtained without issue. As touched upon previously, the pH levels of the water should be around 6-8-7.6. If you obtain readings that are higher or lower than this range, you'll likely need to treat the water or replace it altogether if you want your fish to adjust to their surroundings and get acclimated to their new environment.

[25] "SEDIMENT-Related Criteria for Surface Water Quality," Available: https://19january2017snapshot.epa.gov/sites/production/files/2015-10/documents/sediment-appendix3.pdf .

Turbidity from other than natural sources shall be restricted to not exceed the following numerical limits:

Cool Water Aquatic Community/Trout Fisheries: 10 Nephelometric Turbidity Units (N.T.U.)

Lakes: 25 N.T.U.

Other surface waters: 50 N.T.U..

[26] . *5.5 Turbidity | Monitoring & Assessment | US EPA*. Available: https://archive.epa.gov/water/archive/web/html/vms55.html.

A turbidity meter consists of a light source that illuminates a water sample and a photoelectric cell that measures the intensity of light scattered at a 90 angle by the particles in the sample. It measures turbidity in nephelometric turbidity units or NTUs. Meters can measure turbidity over a wide range from 0 to 1000 NTUs. A clear mountain stream might have a turbidity of around 1 NTU, whereas a large river like the Mississippi might have a dry-weather turbidity of around 10 NTUs. These values can jump into hundreds of NTU during runoff events. Therefore, the turbidity meter to be used should be reliable over the range in which you will be working Meters of this quality cost about $800. Many meters in this price range are designed for field or lab use.

[27] . *WATER 3.ESTIMATES OF WATER FLOW*. Available: https://www.fao.org/fishery/static/FAO_Training/FAO_Training/General/x6705e/x6705e03.htm.

TABLE 3
Water flow measurement methods for streams or canals

Section	Method	Water flow	Accuracy	Remarks	Equipment
3.1 *	Quick and rough	Small	An approximation	For a quick estimate	None
3.2**	Bucket	Very small	Very high	Most accurate of all methods	Dam, pipe, buckets, 1 -l bottle, watch
3.3**	Float	Small to large	Medium	Best for streams with calm water	Float, stakes, line, measuring stick, watch
3.4**	Float and cross section		Low to medium		Float, stakes, line, measuring stick, record sheet, watch
3.5**	Dye, stain and cross section				Dye, stakes, line, measuring stick, record sheet, watch
3.6***	Weir, triangular	Does not vary greatly, 114 l/s or smaller, or does vary greatly from small to large	High	For recording flow over a period of time	Wood, sheet metal or corrugated roof sheeting; tools for working with wood or metal; shovel, pick, line, level, measuring stick
	Weir, rectangular	Does not vary greatly and is greater than 114 l/ s			

[28] "Water Flow for Adequate Filtration in Freshwater and Saltwater Aquariums," 2020. Available: https://www.seatechh2o.com/water-flow-for-adequate-filtration-freshwater-and-saltwater-aquariums/.

Generally, an ideal flow rate is four times the size of your tank. For example, if you have a 30-gallon tank, the recommended water flow for adequate filtration would be 120 gallons per hour (GPH).

[29] L. Admin. (-02-22). *Kelvin Color Temperature Chart | Lighting Color Scale at Lumens*. Available:

https://www.lumens.com/the-edit/the-guides/understanding-kelvin-color-temperature/.

Color Temperatures of Light Bulbs

- **Less than 2000K:** gives off a dim glow of light, similar to what you might find from candlelight; best for low-light areas where ambient illumination is welcomed.
- **2000K-3000K:** gives off a soft white glow, often yellow in appearance; best for living rooms, dining rooms, bedrooms and outdoor spaces.
- **3100K-4500K:** gives off a bright amount of white light; best for kitchens, offices, work spaces and vanities where task lighting is needed.
- **4600K-6500K:** gives off a bright amount of blue-white light, similar to that of daylight; best for display areas and work environments where very bright illumination is needed.
- **6500K and up:** gives off a bright bluish hue of light, often found in commercial locations; best for task lighting.

[30] "Aquarium lighting basics | Tetra®," Available: https://www.tetra-fish.com/learning-center/get-educated/aquarium-lighting-basics.aspx.

Choose the right bulbs: For shallow freshwater tanks, bulbs rated around 5,500 Kelvin work well. For deeper or marine tanks, you might need higher Kelvin ratings, up to 20,000K.

[31] Jacob W. Brownscombe *et al*, "Foraging Behaviour and Activity of a Marine Benthivorous Fiish Estimated using tri-axial accelerometer biologgers." vol. . 505: 241–251, 2014, 2014. Available: https://www.int-res.com/articles/meps2014/505/m505p241.pdf. DOI: Vol. 505: 241–251, 2014 doi: 10.3354/meps10786.

Wetland mesocosm

Five bonefish (45.6 ± 2.7 cm FL_i; 1141 ± 173 g) were equipped with accelerometer loggers in the same manner as the swim tunnel study and were released immediately after tagging into an ~2500 m^2 enclosed wetland mesocosm in Eleuthera on 22 February 2012. The wetland is situated adjacent to the sea and receives a constant influx of seawater from a wetlab facility. It is vegetated with red mangrove *Rhizophora mangle*, black mangrove *Avicennia germinans*, and *Halimeda* spp. Tagged bonefish remained in the wetland for 5 d, during which time behavioural observations were conducted by an observer from a raised bridge that runs through the wetland for 2 h in the morning (07:00 to 09:00 h), 1 h in the afternoon (12:00 to 14:00 h), and 2 h in the evening (16:30 to 18:30 h). The observer noted the timing of behaviours including swimming, burst swimming, coasting, resting, and foraging using the same watch-logger synchronization as the swim tunnel study. Individual bonefish were identified by unique colouration of each accelerometer. A temperature logger (Thermochron iButton ver. DS1921G, –40 to 80°C range, Maxim) was placed in the center of the wetland for the course of the study.

[32] "Biological evaluation of medical devices -- Part 2: Animal welfare requirements ISO 10993-2:2022," Available: https://online-viewer-techstreet-com.myaccess.library.utoronto.ca/virtualviewer/launchViewer.jsp.

This document sets out essential requirements that safeguard animal welfare by minimizing the pain and distress caused when animal tests are considered or undertaken by:

— establishing a framework that reflects the relevant ethical and, in many jurisdictions, the legal considerations relating to the use of animals for experimental or other scientific purposes;

— minimizing the number of animal tests by the appropriate use of literature searches, data-sharing, validated replacement alternatives, and appropriate testing strategies and study designs;

— minimizing any pain, suffering, distress and lasting harm caused to animals used in tests to evaluate the biocompatibility of materials used in medical devices by requiring appropriate use of relevant reduction and refinement alternatives;

— promoting consistent, high standards of accommodation and care to safeguard both the welfare of the animals used and the scientific validity and the reproducibility of the data generated;

— appropriate veterinary care program overseen by a qualified laboratory animal veterinarian is implemented.

4.2 Justification for animal tests

When required to make proper provision to ensure human safety, animal testing to enable the proper biological characterization of materials used in medical devices is acceptable.

For the purposes of the ISO 10993 series, animal tests shall only be deemed to be justified when:

— the resulting data are not otherwise available, but are essential to properly characterize the test material in the context in which it is to be used;

— when no suitable scientifically validated test method not involving the use of living animals is reasonably and practically available;

— when relevant reduction and refinement strategies have been identified and implemented including, if appropriate, obtaining test data from manufacturers and suppliers, and literature searches for toxicity and biocompatibility data.

To avoid unnecessary duplication, before animal tests to evaluate the biocompatibility of materials used in medical devices are undertaken, a review of available, relevant information on the properties of the test material shall be undertaken and documented. This shall include taking reasonable steps to enable data sharing.

Animal tests are deemed to be justified only when:

— they have been shown to be relevant and reliable for the purposes for which they are undertaken;

— the resulting data are essential to properly characterize and evaluate the test material in the context in which it is to be used in medical devices;

— no scientifically valid test method not requiring the use of living animals is reasonably and practically available;

— other relevant and appropriate strategies to minimize the pain, suffering, distress and lasting harm caused to the animals that are used have been identified and implemented.

4.3 Competence of personnel

Animal tests shall be designed, conducted and interpreted by persons competent to discharge the responsibilities assigned to them.

Animal tests shall be designed and conducted with the involvement of personnel with expertise in veterinary science, laboratory animal science and medicine, and animal husbandry and care.

Details of how staff are equipped by experience, qualification and training (including continued professional development) to satisfy these requirements shall be documented.

NOTE 1 Although this document does not provide an objective specification, it is considered important that those involved in the conduct of animal tests demonstrate a caring and respectful attitude to the animals used, i.e. that they have an appropriate "culture of care".

NOTE 2 For further information on assurance of training and competency, see 7.8.5 of Reference [1].

4.6.1 General

Purpose-bred animals shall be used whenever possible and specific justification is required for the use of non-purpose bred animals.

When purpose-bred animals are not used, the justification and details of the origin or source of the animals that are used shall be documented.

High standards of care and accommodation enhance the welfare of the animals used and promote the scientific validity of animal testing. Animal care and accommodation shall demonstrably, as a minimum, conform to relevant relevant national or regional regulations, published national or international animal care, accommodation and husbandry guidelines.

The relevant guidelines or requirements shall be referenced, and evidence of compliance (or details of non-compliance accompanied by an assessment of its likely impact on the welfare of the animals used and the validity of the data obtained) shall be explained, justified and documented.

Any component of the husbandry system that does not make best provision for the welfare of the test animals, can compromise the scientific validity of the test or inappropriately influence the nature or interpretation of the test result, shall be documented.

Social species shall be housed as stable, compatible pairs or groups unless single-housing is required for veterinary, husbandry, animal welfare or scientific reasons.

When it is not possible to pair- or group-house social species, the veterinary, husbandry, animal welfare or scientific justification for the need for single housing and its duration shall be documented. The impact of the decision made on the scientific outcome should also be evaluated and documented.

Custom and practice shall not, of themselves, be deemed to be acceptable justifications.

[33] "Transporting Fish," pp. 6–8, Available: https://cales.arizona.edu/azaqua/AquacultureTIES/publications/English%20WHAP/GT1%20Transp.pdf.

Table 1: Quantities of different sized fish that can be transported in sealed plastic bags (18 inch x 32 inch) with approximately 7.6 liters of water and pure oxygen.

	Duration of Transport			
Fish Size	1 HR	12 HR	24 HR	48 HR
Newly Hatched Larvae (grams/l)	120	80	40	10
1/4 inch (0.64 cm) Fry (grams/l)	60	50	40	20
1 inch (2.54 cm) Fingerling (grams/l)	120	100	75	40
2 inch (5.08 cm) Fingerling (grams/l)	120	105	90	40
3 inch (7.62 cm) Fingerling (grams/l)	120	105	90	40
Larger Fish (grams/l)	480	180	120	60

Table 2: The weight of fish in grams per liter of water transported in tanks with diffused oxygen.

	Duration of Transport			
Fish Size	1 HR	6 HR	12 HR	24 HR
Larvae and Fry	NR*	NR*	NR*	NR*
1 inch Fingerling	120	60	30	30
2 inch Fingerling	240	180	120	120
3 inch Fingerling	360	240	120	120
8 inch Fingerling	360	360	240	180
Larger Fish	480	480	360	240

* NR = Not Recommended

[34] "Electric Aquarium Equipment UL 1018," 2011. Available: https://subscriptions-techstreet-com.myaccess.library.utoronto.ca/products/596481.

[35] "Packaging -- Transport packaging for dangerous goods -- Test methods ISO 16495:2022," Available: https://online-viewer-techstreet-com.myaccess.library.utoronto.ca/virtualviewer/launchViewer.jsp.

F.3.1 General

The tests shall be carried out in accordance with ISO 2248:1985, except for 7.2, the drop heights, drop attitude and number of samples to be tested shall be determined from the UN Recommendations. For drops performed on packaging other than flat drops, the centre of gravity shall be vertically over the point of impact.

Prior to carrying out the test, ensure that the drop area is clean and dry.

ISO 2248:1985, 4.4, does not apply. Impact surfaces shall be in accordance with the UN Recommendations.

NOTE For packaging, a suitable impact surface can have a mass at least 50 times the mass of the test item and have a maximum length of 5 times of its thickness. This note does not apply to IBCs or large packaging.

F.3.2 Information drops for packaging

To assess the weakest point, information drops may be performed. Where such investigation drops are undertaken they may be done with packaging already used in other tests. Each packaging shall strike the target in an orientation designed to investigate the weakest part. The orientations to be taken into account vary with designs. Information drops should be used to seek an alternative orientation where the design is not a common design.

inner packaging or articles undergoes a drop test, the combination packaging/large packaging shall pass the test if the entire contents are retained by the inner packaging or inner receptacle (e.g. plastic bag) even if the closure is no longer sift proof. If there is dampness in the dropping area, the combination packaging/large packaging shall be moved carefully to a suitable place for examination of any leakage that may occur (e.g. moved so that it is on a surface such as clean fibreboard where drips should be apparent). Examination shall last up to 5 min. Where a combination packaging/large packaging undergoes a cold drop test, immediately after dropping the first combination packaging/ large packaging, the temperature of the package and/or its contents shall be checked and recorded in the test report. Subsequent packaging does not need the temperature checked unless the first sample had not achieved the required temperature.

G.4 Method of assessment

Observation should be made for the escape of air bubbles throughout the test.

Air bubbles considered to arise from entrapped air (e.g. air held initially in seams or in the thread of closures) cannot be considered as leakage: these include any bubbles which do not appear regularly or produced at intervals exceeding 1 min. If necessary, the test period should be extended to allow entrapped air to be expelled.

I.3 Calculation of the stacking load for packaging

I.3.1 General

In the following calculations, where the design type has an interstacking feature, an allowance shall be made. This usually takes the form of a small reduction in effective packaging height.

I.3.2 Solids, articles, or liquids to be transported

Where the contents are solids, articles, or the liquid to be transported, the stacking load to be superimposed on each packaging shall be calculated as given by Formula (I.1):

$$m_1 = \left(\frac{H}{h} - 1\right) \cdot m_{p,f,c} \qquad (I.1)$$

where

m_1 is the stacking load in kilograms (kg) (with closure included; see NOTE);

$m_{p,f,c}$ is the mass in kilograms (kg) of the complete, filled and closed packaging as prepared for transport;

H is the relevant stack height in millimetres (mm);

h is the overall height in millimetres (mm) of packaging to be tested, allowing for any inter-stacking features.

NOTE Newtons can be used as a unit of force.

I.5 Method of assessment

Observation should be made for leakage and deformation of packaging, inner packaging, large packaging and IBCs (excluding flexible IBCs).

Observation should be made for deterioration of the body and leakage of flexible IBCs.

A suitable method for guided loads is as follows. The packagings are removed from the stack rig. For stackable packaging, two filled of the same type should be placed centrally on the tested packaging. For not individually stackable packaging the stacked samples may be arranged as Figure I.1 on which two identical layers are placed on top using intermediate plates of suitable material. In both cases the packaging should maintain their position for one hour.

Where unguided loads have been used, this may be assessed by the angle of the top plate Anan angle of 5° or more may be considered to show significant deformation. The 5° criterion has been found to accord with the UN requirements in relation to stack stability.

Tear test

M.1 Applicability

The tear test is for all types of flexible intermediate bulk containers (FIBCs).

M.2 Preparation

Samples for this test shall be prepared in accordance with the UN Recommendations and the relevant parts of Clause 4.

M.3 Test method

The test sample shall be tested as determined by the UN Recommendations.

M.4 Method of assessment

Observation should be made for propagation of the cut.

[36] "Packaging -- Complete, filled transport packages -- Rolling test ISO 2876:1985," Available: https://online-viewer-techstreet-com.myaccess.library.utoronto.ca/virtualviewer/launchViewer.jsp.

Conditioning for testing.

3 Principle

Rolling of the test package so as to impact on each face in turn.

4 Apparatus

Impact surface, horizontal and flat, massive enough to be immovable and rigid enough to be non-deformable under test conditions.

NOTE — In normal circumstances the impact surface provided shall be

— integral with a mass at least 50 times that of the heaviest package to be tested;

— flat, such that no two points on its surface differ by more than 2 mm; however, where one of the dimensions of the test package in contact with the surface is greater than 1 000 mm, a maximum difference in surface level of 5 mm will be acceptable;

— rigid, such that it will not be deformed by more than 0,1 mm when an area of 100 mm^2 is loaded statically with 10 kg anywhere on the surface;

— sufficiently large to ensure that the test package falls entirely upon the surface.

7 Procedure

Whenever possible the test shall be carried out in the same atmospheric conditions as used for conditioning, where this is critical to the materials or application of the package. In other circumstances, the test shall be carried out in atmospheric conditions which are as near as practicable to those used for conditioning.

7.1 Parallelepipedal packages

Define the panels and edges of the test package using the requirements given in ISO 2206.

Place the package on the impact surface (see clause 4) with surface 1 uppermost.

Tilt the package by hand with the edge 3-4 resting on the impact surface until the point of balance on this edge is reached. Then permit it to overbalance without thrust so as to impact on surface 4.

Repeat this procedure until the sequence given in the table is completed.

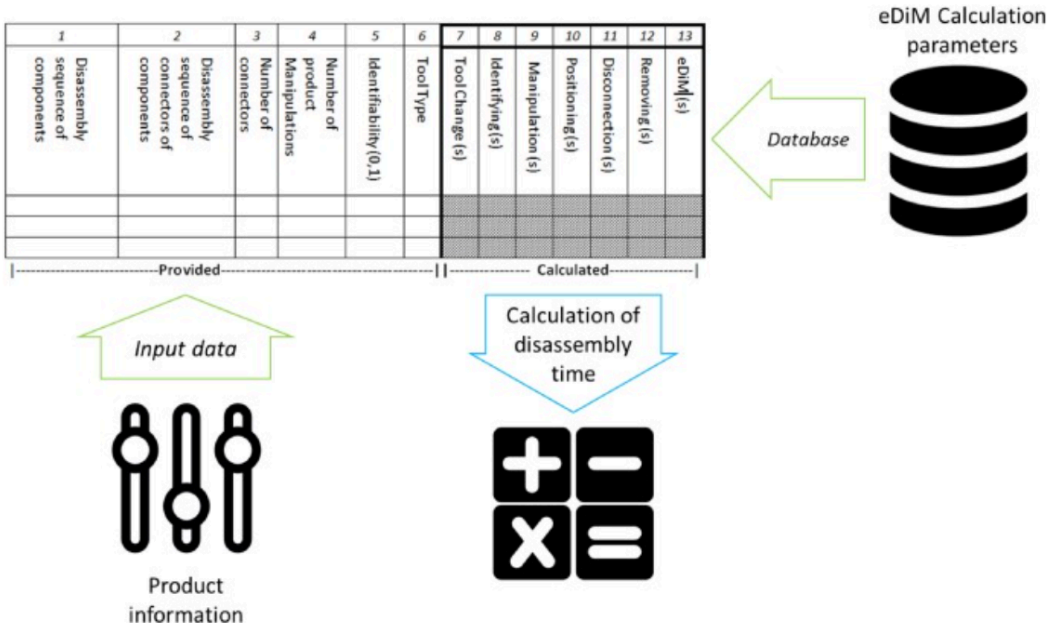
[37] L. Swann. . *Transportation of Fish in Bags*. Available: https://thefishsite.com/articles/transportation-of-fish-in-bags.

Bag shipment requires placing a prescribed weight of fish in 1.5 to 2 gallons of water in 3 ml polyethylene bags, 18 by 32 inches. Excess air is removed from

Stage or total length in inches		Carrying capacities (pounds) for transport period in hours			
		1	12	24	48
Eggs		1.0-3.0	1.0-2.0	1.0-1.5	0.5-1.0
Fry	Yolk-sac	2.0-4.0	1.4-3.0	0.8-2.0	0.2-1.5
	Swim-up	1.0-4.0	0.9-3.0	0.8-2.0	0.4-1.4
Fingerlings	1/2	1.8-5.0	1.5-4.0	1.2-3.0	0.6-1.5
	1	2.0-5.0	1.7-4.0	1.3-4.0	0.7-2.0
	2	2.0-7.0	1.8-6.0	1.5-4.0	0.7-2.0
	3	2.0-7.0	1.8-6.0	1.7-4.0	0.7-2.0
Large fish		4.0-9.0	3.0-6.5	2.0-5.0	1.0-2.5

[38] P. Vanegas *et al*, "Ease of disassembly of products to support circular economy strategies," *Resour. Conserv. Recycling,* vol. 135, pp. 323–334, 2018. Available: https://www.sciencedirect.com/science/article/pii/S0921344917301763. DOI: 10.1016/j.resconrec.2017.06.022.

Table 4. Disassembly time calculation sheet.



$$eDiM = \sum_{i=1}^{i=n} (Tool\ Change_i + Identifiying_i + Manipulation_i + Positioning_i + Disconnection_i + Removing_i) \quad (1)$$

(in seconds)

[39] C. Favi *et al*, "Big data analysis for the estimation of disassembly time and de-manufacturing activity," *Procedia CIRP,* vol. 90, pp. 617–622, 2020. Available: https://www.sciencedirect.com/science/article/pii/S2212827120301608. DOI: 10.1016/j.procir.2020.01.072.

Disassembly time is one of the most important metrics to guide the implementation of DfD strategies and thus to adopt CE models (Mandolini et al., 2018). Disassembly time depends by several factors, such as component shape, joining elements, disassembly directions, disassembly tools and equipment (Güngör, 2006). It is influenced by the product workload (life cycle stress), working environment, chemical and physical degradation (ageing), deformation, cleanliness, material type, coating/painting processes, etc. (Duflou et al., 2008). The condition of the product and its constituent components could be uncertain when disassembly occurs, and this kind of information needs to be processed systematically in order to develop any realistic and credible disassembly plan (Zhu and Roy, 2015). Thus, disassembly time is not the reverse of assembly time and its estimation requires the analysis of real de-manufacturing operations performed in different contexts (e.g. dismantling centres and/or service centres) (Sodhi et al., 2004). This issue has been faced with the aim to collect all this information in a structured way (Favi et al., 2016).

Table 1. Corrective factors for "nut" liaison. (*) standard conditions.

Corrective factor type	Corrective factor condition	Average disassembly time [s]	Corrective factors [s]
Unscrewing tool	Plier	10.20	1.88
	Screw driver*	**5.42**	**1.00**
	Wrench	6.96	1.28
	Reference	*5.42*	
Head type	**Hexagonal***	**6.89**	**1.00**
	Hexagonal self-locking	7.50	1.09
	Hexagonal domed cap	7.55	1.10
	Wing	8.16	1.18
	Reference	*6.89*	
Length	High	8.27	1.22
	Medium	7.51	1.11
	Short*	**6.79**	**1.00**
	Reference	*6.79*	
Diameter (mm)	4	8.04	1.16
	7	7.72	1.11
	12*	**6.93**	**1.00**
	Reference	*6.93*	

(sample aspects that can be taken into consideration)

[40] "Environmental management - Integrating environmental aspects into product design and development ISO/TR 14062:2002," Available: https://subscriptions-techstreet-com.myaccess.library.utoronto.ca/products/176198.

Use of a multi-criteria concept

Using the life cycle considerations in 7.2.3, different approaches may be applied, such as

— reduction in product mass or volume,

— improvement in energy efficiency,

— lengthening of product life,

— choice of materials and processes used.

Applying and combining these criteria may reduce environmental impacts of the product. For example:

— A reduction in product mass or volume may be the result of optimizing material use, thereby reducing impacts associated with resource depletion. The reduced product mass or volume could decrease shipping mass or volume, thereby reducing emissions associated with transport.

— Improving energy efficiency, both during the use of the product and in standby mode, is important, particularly in domestic electrical appliances.

— A longer product life may decrease requirements for resources. Extending product life can however delay the implementation of technological advances that may improve the environmental performance.

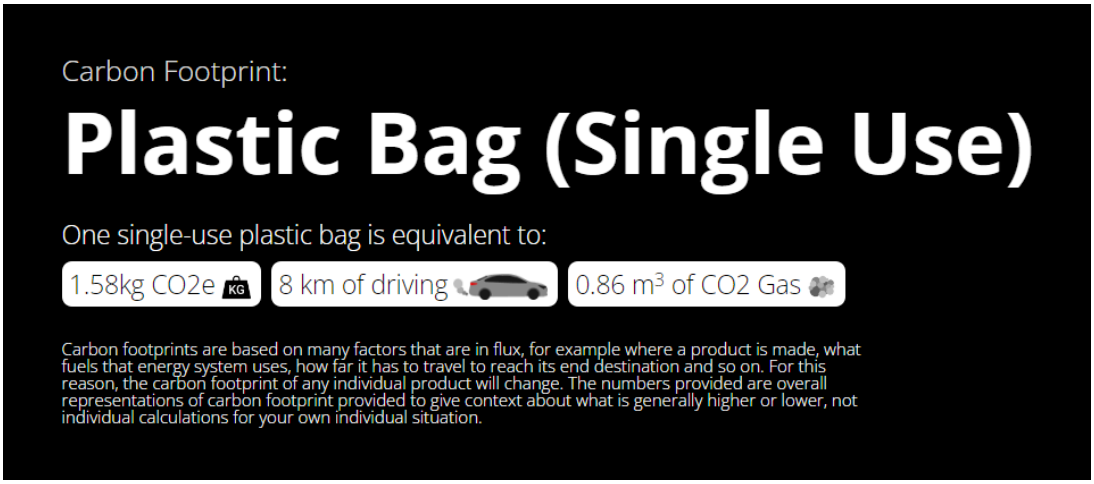
— Designing products to facilitate disassembly can extend product life through reuse of product parts and can encourage recycling.

[41] Legacy Winnipeg, "Emission factors in kg CO2-equivalent per unit," Available: https://legacy.winnipeg.ca/finance/findata/matmgt/documents/2012/682-2012/682-2012_appendix_h-wstp_south_end_plant_process_selection_report/appendix%207.pdf.

Material	Iron metal	Iron	Cast iron	kg	1.51	
Material	Iron metal	Iron	Iron	kg	1.91	
Material	Iron metal	Steel	Reinforced steel	kg	1.49	
Material	Iron metal	Steel	Stainless steel	kg	6.15	
Material	Iron metal	Steel	Steel	kg	1.77	
Material	Iron metal	Steel	Steel r.	kg	0.88	10%
Material	Iron metal	Steel	Steel v.	kg	3.29	10%
Material	Non Iron metal	Aluminium	Aluminium	kg	8.14	
Material	Non Iron metal	Aluminium	Aluminium r.	kg	2.01	30%
Material	Non Iron metal	Aluminium	Aluminium v.	kg	11.89	30%
Material	Non Iron metal	Copper	Copper	kg	2.77	50%
Material	Non Iron metal	Copper	Copper v.	kg	3.83	
Material	Non Iron metal	Glass	Glass	kg	0.85	
Material	Non Iron metal	Glass	Glass r.	kg	0.73	20%
Material	Non Iron metal	Glass	Glass v.	kg	4.40	20%
Material	Non Iron metal	Lead	Lead	kg	1.64	30%
Material	Non Iron metal	Lead	Lead r.	kg	0.53	
Material	Non Iron metal	Lead	Lead v.	kg	2.61	
Material	Non Iron metal	Nickel	Nickel	kg	11.53	30%
Material	Non Iron metal	Other	Other metal	kg	4.40	80%
Material	Non Iron metal	Zinc	Zinc	kg	3.41	20%

(sample emissions for materials in 2nd column from right)

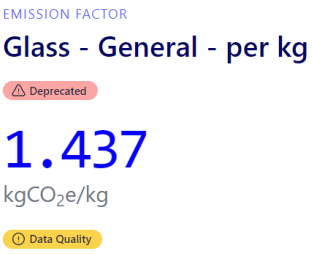
[42] . *Plastic Bag (Single Use) Carbon Footprint | 1.58kg CO2e*. Available: https://www.co2everything.com/co2e-of/plastic-bag.



[43] K. Williamson. . *Carbon Footprint of Fish Tanks: Emissions Guide For Fish Tanks By Size, Type*. Available: https://8billiontrees.com/carbon-offsets-credits/carbon-footprint-of-fish-tanks/.

Worldwide glass production was responsible for 95 million Mt (metric tons) in 2022.[6] Acrylic (PMMA) is a type of synthetic plastic, and sources suggest that every kg of PMMA produced generates 5.5 kg of CO_2e.[13]

[44] Climatiq. . *Glass - General - per kg*. Available: https://www.climatiq.io/data/emission-factor/9ab3d163-4759-480a-b181-0aa84d994fc1.



[45] "Ergonomics Data for use in the application of ISO/IEC Guide 71:2014, ISO/TR 22411:2021," 2014. Available: https://online-viewer-techstreet-com.myaccess.library.utoronto.ca/virtualviewer/launchViewer.jsp.

[46] "Ergonomics of human-system interaction ISO 9241-11:2018," Available: https://subscriptions-techstreet-com.myaccess.library.utoronto.ca/products/783901.

6.6.2 Accessibility

Accessibility is the extent to which products, systems, services, environments and facilities can be used by people from a population with the widest range of user needs, characteristics and capabilities to achieve identified goals in identified contexts of use. The aim in designing for accessibility is to widen

the target population, thus making products, systems, services, environments and facilities more accessible to more people in more diverse contexts of use (see A.6.2).

Usability is relevant to:

— regular ongoing use, to enable users to achieve their goals effectively, efficiently and with satisfaction;

— learning, to enable new users to be become effective, efficient and satisfied when starting to use a system, product or service;

— infrequent use, to enable users to be effective, efficient and satisfied, with the system on each reuse;

— use by people with the widest range of capabilities;

— minimizing the risk and the undesirable consequences of use errors; and

— maintenance, in that it enables maintenance tasks to be completed effectively, efficiently and with satisfaction.

Usability is relevant when designing or evaluating interactions with a system, product or service for the purposes of:

— development;

— procurement;

— review or comparison; and

— marketing and market research.

[47] "Ergonomics of human-system interaction - Part 20: An ergonomic approach to accessibility within the ISO 9241 series ISO 9241-20:2021," Available: https://online-viewer-techstreet-com.myaccess.library.utoronto.ca/virtualviewer/launchViewer.jsp.

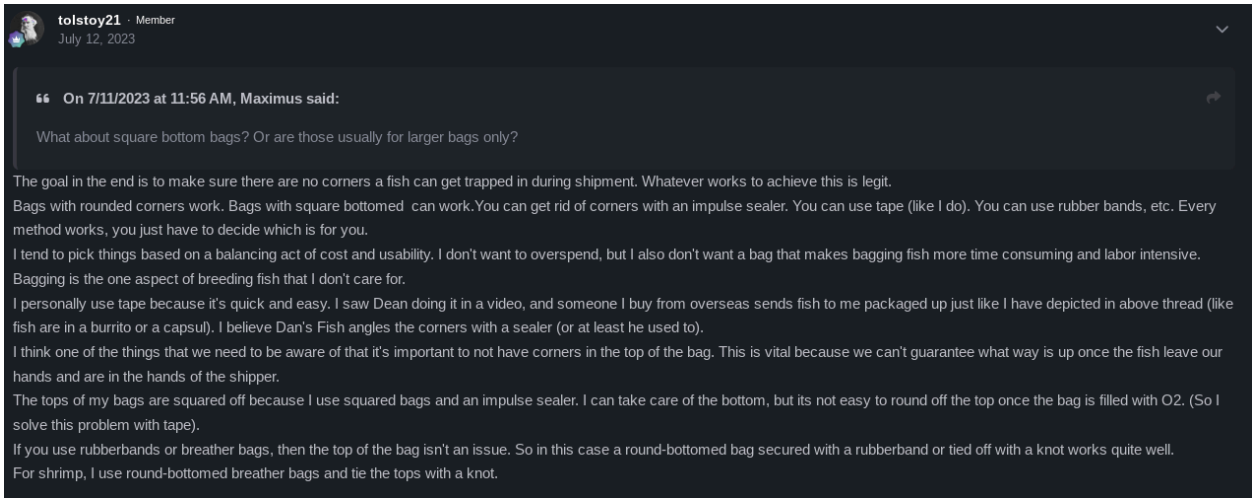
and available to the widest range of users within the intended user population, taking account of their special abilities, variations in their capabilities, the diversity of their tasks, and their differing environmental, economic and social circumstances."

NOTE ISO/IEC Guide 71:2014, 6.2.1 transforms this principle into a goal it calls "suitability for the widest range of users". It explains that "A system is suitable for the widest range of users if it meets the needs of diverse users in diverse contexts."

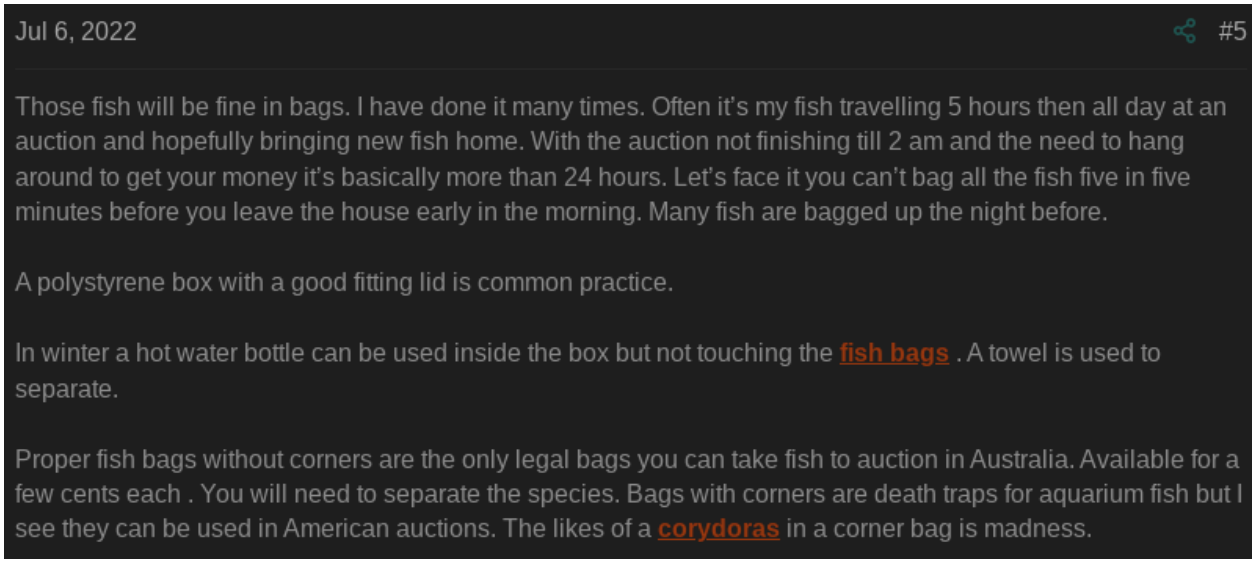
ISO 9241-11:2018, 3.1.7, defines a user as a "person who interacts with a system, product or service". It recognizes that, "the objective of designing for accessibility is to enable products, systems, services, environments and facilities to be used by people with the widest range of user needs, characteristics and capabilities in diverse contexts of use. Accessibility is included as a component of human-centred quality to emphasize its importance as part of human-centred design."

While the accessibility guidance in ISO 9241-171 and ISO 9241-971 can support suitability for the widest range of users, there are no parts of the ISO 9241 series that provide guidance on identifying the widest range of users.

[48] (-07-09). *Best fish bags?*. Available: https://forum.aquariumcoop.com/topic/33534-best-fish-bags/.



[49] (-07-05). *How do I transport 9 nine fish on a 8 hour drive | Freshwater Aquarium Discussion Forum*. Available: https://www.fishlore.com/aquariumfishforum/threads/how-do-i-transport-9-nine-fish-on-a-8-hour-drive.524525/.



[50] C. Watson, K. H. Kilgore and C. Martinez, "Shipping Fish in Boxes," Available: https://srac.msstate.edu/pdfs/Fact%20Sheets/3903%20Shipping%20Fish%20in%20Boxes.pdf.

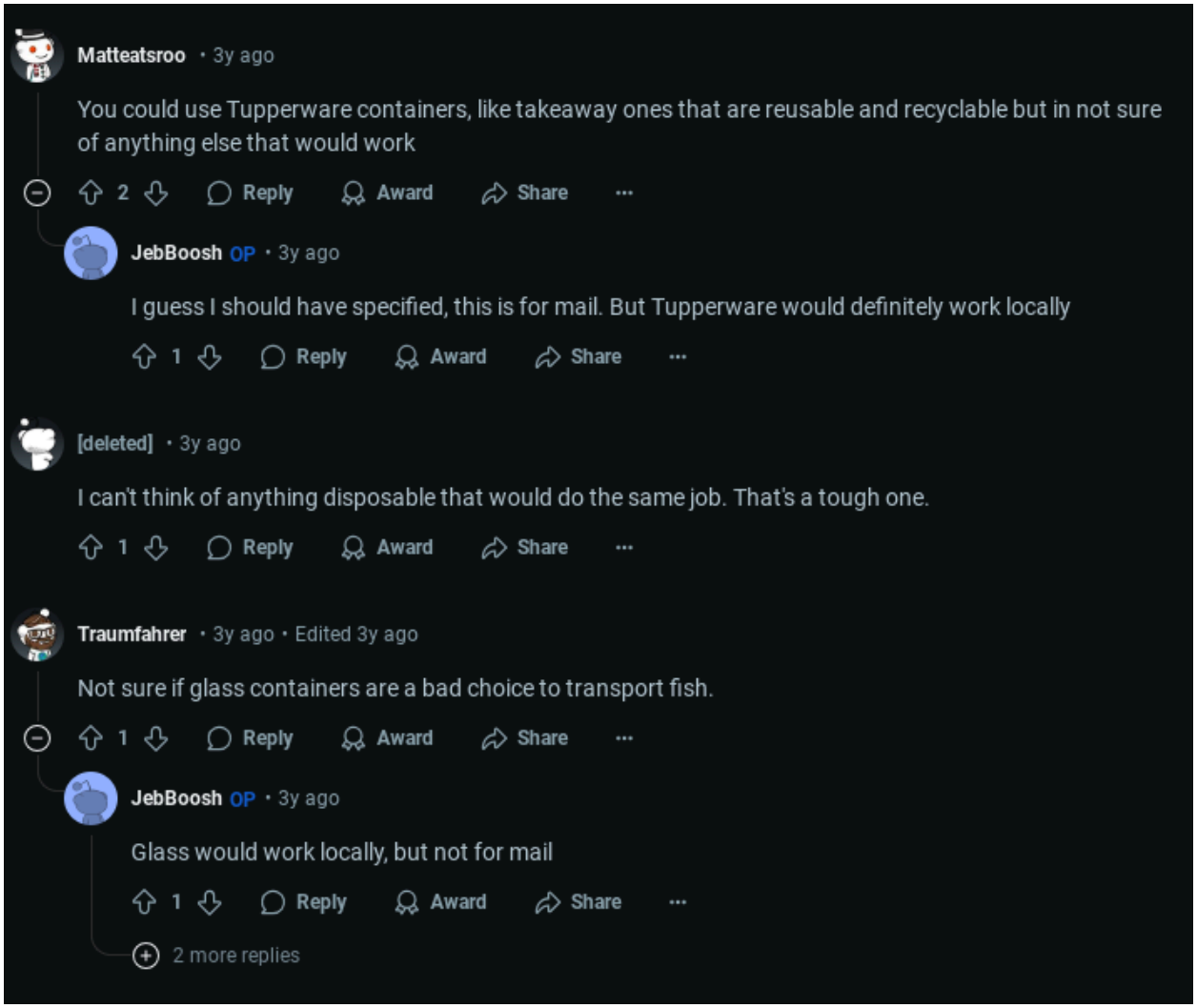
Outer shipping box

While Styrofoam® is watertight and provides good insulation, it is not a rugged material and can be easily punctured, chipped or broken during the shipment. So the insulated box must be placed in a rugged outer container. Usually this is a corrugated cardboard box that will protect the Styrofoam® or other insulation from damage. Like bags and Styrofoam® boxes, outer cardboard boxes specifically designed for shipping fish are available from several Internet sources. The inner, insulated box should fit snuggly inside the outer box to give the best protection possible. A good quality packing tape should be used to seal the outer box.

[51] R. Bremner, "Bagging Your Fish for the Auction | Calgary Aquarium Society," 2010. Available: https://www.calgaryaquariumsociety.com/bagging-your-fish-for-the-auction/.

- Allow for a ratio of approximately **75% air / 25% clean tank water** in each bag for maximum oxygen levels and to keep your fish comfortable. The water should cover your fish while the bag is on its side... keep in mind *oxygen is more important than water*. At this point you could optionally add a small amount of conditioner (or de-stressor) to the water.

[52] JebBoosh, "Sustainable alternative to plastic fish bags?" 2022. Available: https://www.reddit.com/r/Aquariums/comments/pnx4mt/sustainable_alternative_to_plastic_fish_bags/.



[53] G. S. Hilmarsdóttir *et al*, "Environmental impacts of different single-use and multi-use packaging systems for fresh fish export," *Journal of Cleaner Production,* vol. 447, pp. 141427, 2024. Available: https://www.sciencedirect.com/science/article/pii/S0959652624008758. DOI: 10.1016/j.jclepro.2024.141427.

Production of the tubs and boxes		EPS	6 kg PE 41 L	42kg PUR 460 L	50 kg PE 460 L		44 kg PE 460 L				38 kg PE Twin 290 L	
Impact category			100/0		100/0	90/10	100/0	90/10	70/30	50/50	100/0	90/10
Abiotic depletion	kg Sb eq	8.2E-02	2.9E-01	1.6E-01	1.8E-01	1.4E-01	1.5E-01	1.2E-01	6.5E-02	9.7E-03	1.8E-01	1.9E-01
Abiotic depletion (fossil fuels)	MJ	2.6E+06	1.6E+06	8.3E+05	9.5E+05	7.6E+05	7.8E+05	6.3E+05	3.2E+05	3.8E+03	9.8E+05	9.3E+05
Global warming (GWP100a)	**kg CO2 eq**	**1.3E+05**	**4.5E+04**	**2.4E+04**	**2.7E+04**	**2.2E+04**	**2.3E+04**	**1.8E+04**	**9.2E+03**	**2.9E+02**	**2.8E+04**	**3.0E+04**
Ozone layer depletion (ODP)	**kg CFC-11 eq**	**3.4E-03**	**6.9E-04**	**3.6E-04**	**4.1E-04**	**3.3E-04**	**3.4E-04**	**2.7E-04**	**1.3E-04**	**-1.1E-05**	**4.3E-04**	**6.4E-04**
Human toxicity	kg 1,4-DB eq	2.5E+04	1.4E+04	7.2E+03	8.3E+03	7.0E+03	6.8E+03	5.7E+03	3.5E+03	1.5E+03	8.5E+03	1.2E+04
Fresh water aquatic ecotox.	kg 1,4-DB eq	2.0E+04	6.0E+03	2.4E+03	2.7E+03	5.0E+03	2.2E+03	4.1E+03	7.6E+03	1.2E+04	2.8E+03	9.3E+03
Marine aquatic ecotoxicity	kg 1,4-DB eq	6.8E+07	2.3E+07	1.2E+07	1.3E+07	2.7E+07	1.1E+07	2.3E+07	4.5E+07	6.9E+07	1.4E+07	2.4E+07
Terrestrial ecotoxicity	kg 1,4-DB eq	1.1E+02	2.5E+01	1.3E+01	1.5E+01	1.4E+01	1.3E+01	1.2E+01	1.0E+01	8.7E+00	1.6E+01	2.4E+01
Photochemical oxidation	kg C2H4 eq	1.9E+02	1.4E+01	7.6E+00	8.6E+00	6.9E+00	7.1E+00	5.7E+00	2.8E+00	-3.6E-02	8.9E+00	8.8E+00
Acidification	**kg SO2 eq**	**4.5E+02**	**1.7E+02**	**9.0E+01**	**1.0E+02**	**8.2E+01**	**8.4E+01**	**6.7E+01**	**3.4E+01**	**6.2E-01**	**1.1E+02**	**1.0E+02**
Eutrophication	kg PO4 eq	8.1E+01	2.9E+01	1.5E+01	1.7E+01	1.3E+01	1.4E+01	1.1E+01	4.4E+00	-1.9E+00	1.8E+01	2.5E+01

Yearly usage		41 L	41 L	460 L	460 L	460 L	290 L
Impact category		EPS	6 kg PE	42 kg PUR	50 kg PE	44 kg PE	38 kg PE Twin
Abiotic depletion	kg Sb eq	3.3E-02	2.3E-03	3.2E-02	3.6E-02	3.0E-02	3.6E-02
Abiotic depletion (fossil fuels)	MJ	8.8E+04	2.0E+03	1.1E+05	1.2E+05	9.9E+04	1.2E+05
Global warming (GWP100a)	**kg CO2 eq**	**6.8E+03**	**3.4E+02**	**8.5E+03**	**9.6E+03**	**8.0E+03**	**9.8E+03**
Ozone layer depletion (ODP)	**kg CFC-11 eq**	**1.1E-03**	**2.0E-05**	**1.3E-03**	**1.5E-03**	**1.2E-03**	**1.6E-03**
Human toxicity	kg 1,4-DB eq	2.6E+03	1.8E+02	3.4E+03	3.8E+03	3.2E+03	3.9E+03
Fresh water aquatic ecotox.	kg 1,4-DB eq	2.0E+03	5.8E+02	2.8E+03	3.0E+03	2.6E+03	2.8E+03
Marine aquatic ecotoxicity	kg 1,4-DB eq	3.4E+06	2.8E+05	3.8E+06	4.3E+06	3.6E+06	4.3E+06
Terrestrial ecotoxicity	kg 1,4-DB eq	7.9E+00	2.0E+02	3.1E+02	3.2E+02	2.9E+02	2.2E+02
Photochemical oxidation	kg C2H4 eq	3.4E+00	1.5E-01	4.7E+00	5.3E+00	4.4E+00	5.4E+00
Acidification	**kg SO2 eq**	**1.2E+02**	**1.5E+00**	**1.7E+02**	**1.9E+02**	**1.6E+02**	**2.0E+02**
Eutrophication	kg PO4 eq	1.8E+01	4.1E+00	2.8E+01	3.1E+01	2.6E+01	3.0E+01

[54] . *Kordon Breathing Bags*. Available: https://www.guppyfarmuk.com/articles-and-information/kordon-breathing-bags.

Kordon ® Breathing Bags™ are a fantastic way of transporting guppies, especially when a flight or extended time is necessary.

The technology was first developed in space/military research and was refined to produce the bags that we have today by Kordon together with plastics & chemical engineers.

The Breathing Bags allow the transfer of gas molecules through the plastic wall of the bag, carbon dioxide and oxygen in particular. As long as there is a breathable atmosphere outside the Breathing Bag, the guppies inside will not run out of oxygen.

Carbon dioxide exits the bags at 4 times the rate oxygen enters the bags, thereby constantly purging the water of toxic carbon dioxide and allowing oxygen to replace it in the water.
Prior to this invention, the only bags available for shipping guppies were made of ordinary polyethylene. When using these "barrier" bags, oxygen must be added as a gas inside the bag prior to sealing and shipment. However, this process has many problems including:
a, high concentrations of oxygen can cause flammable conditions,
b, the presence of oxygen gas inside the bag takes up a lot of valuable shipping space,
c, once the supplied oxygen is used up there is no more available,
d, toxic carbon dioxide from the fishes' breathing builds up in the water, displacing the oxygen
e, the oxygenated air in the bags may not be satisfactory for fishes' breathing, because the bottled oxygen can be contaminated,
f, a bag partially full of water with the rest filled with oxygen allows the contents to slosh during transport, stressing fishes.
After adding water and a guppy to the Breathing Bag, seal the bag with no airspace. I prefer to do this with a knot rather than with an elastic band. Breathing bags can be sealed using all of the current methods: rubber bands, twist ties, metal clips, etc.

[55] *Tips needed for shipping fish in breather bags* Apr. 2017. Available: https://www.plantedtank.net/threads/tips-needed-for-shipping-fish-in-breather-bags.45606/.

METHODS FOR USE:
After adding water and fish, seal the bag with as little airspace as possible.
Except for those few kinds of fishes that are made uncomfortable by the lack of an air space at the surface, fishes adapt readily to the lack of an air space and it is not needed. It is best if there is no air pocket in the bag so that there is no water movement, keeping the fishes calmer. And uneeded air space also uses up valuable shipping space.
Breathing bags can be sealed using all of the current methods: rubber bands, twist ties, metal clips, etc. An excellent way for fast efficient sealing is with a bar type heat sealer. The plastic used in the "Breathing Bags" readily seals with heat. Heat sealing can be done much more quickly than other methods and greatly increases the speed with which bags can be handled and sealed. For those sealing many bags for shipment the change will be a dramatic reduction in labor.
The proper use of certain water conditioners will greatly enhance the effectiveness of the Breathing Bags. Either a combination of AmQuel® and NovAqua® or AmQuel and PolyAqua® with a suitable antibiotic (Kordon has found Neomycin to be among the best of the antibiotics for universal usage) has proven the most effective. All the Kordon "Sachets" have special combinations of additives to increase the well being and survival of the inhabitants. These additives are in the process of being marketed under the general trade name of "Kordon Aquatic Life Saver". There are separate products for freshwater and marine conditions.
CHARACTERISTICS:
The plastic in the "Breathing Bags" is very tough and flexible. The thickness is 1.5 ml for the regular bags and 3 ml for the liner bag. Small punctures such as from fish spines often do not penetrate the plastic, and if they do, the molecular structure of the plastic tends to realign and reduce the size of the holes or reseal itself. Some fishes may damage the bag film enough to cause leaks. Only experimentation and experience will determine which individual species of fishes are safe to transport . Kordon is experimenting with a heavier walled and perforated liner bag to fit inside the Breathing Bag to help with the puncture problem. For most smaller spiny fishes it is sufficient to multiple bag them, Breathing Bag within Breathing Bag, preferably with the inner bag wrapped in one or more layers of newspaper. There is a proportionate loss of breathability (up to approximately 50%) for one bag inside another, which will affect different animals differently. The user should experiment to find acceptable conditions for multiple bagging.

Here is a link if you want to save it to your computer.
http://www.kensfish.com/files/GENERAL_INFORMATION_ON_KORDON.doc Be very careful double bagging breather bags, I believe it advises against it on the bags. Larger fish should be shipped in regular bags, Kenny Bin Laden busted through the breather bag I shipped him in, lack of O2 made him a terrorist. I always squeeze the air out, tie one knot and pull it tight then squeeze for pin holes. Definitely use a water conditioner, I use ship safe and have shipped hundreds of shrimp and Endler's this way; have way less than 1% DOA....DC

[56] Seachem. *Stressguard*. Available: https://www.seachem.com/stressguard.php.

Overview

StressGuard™ is the premium slime coat protection product. StressGuard™ will reduce stress and ammonia toxicity whenever handling or transporting fish. StressGuard™ binds to exposed protein in wounds to promote healing of injured fish and to reduce the likelihood of primary and secondary infections. StressGuard™ is compatible with all medications except those that are copper based. StressGuard™ is not amine based and will not interfere with Purigen®; or HyperSorb™ regeneration. StressGuard™ does not coat resins or clog filtration like competing "slime coat" type products can.

What makes StressGuard™ very successful at healing is the fact that it contains protein active colloids. This protein active colloidal agent actively seeks out any wounds, abrasions, or places where exposed proteins are and attaches to this area to help directly deliver the disinfectant in the product and start the healing process. This protein active colloid, because it binds to the exposed proteins, also acts like a liquid bandage to the abrasion. Some products are marketed to be squirted in the bag when a fish is purchased. Those products are generally acidic, with some brands being a very strong acid. Putting such a product in a small, enclosed container with a fish often does more harm than any intended good. StressGuard™ has a pH of 7.0. It will not affect the pH of an aquarium, even at large doses. There are no other products on the market that can compare to the effectiveness of StressGuard™.

[57] M. Vanderzwalmena *et al*, "Effect of a water conditioner on ornamental fish behaviour during commercial transport," 2019. Available: https://www.sciencedirect.com/science/article/pii/S0044848619313043.

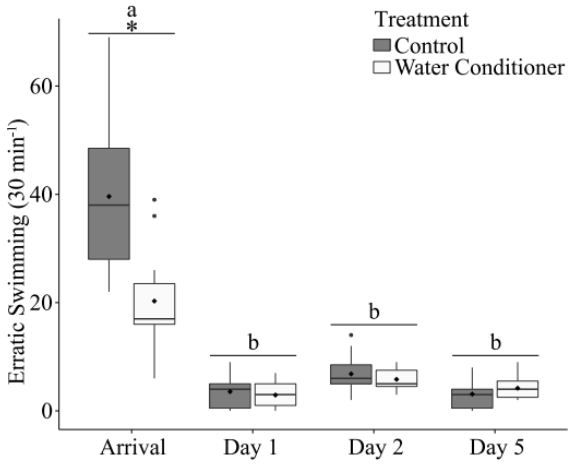


Fig. 2. Erratic swimming behaviour following addition of water conditioner during regional transport. The occurrence of erratic swimming behaviour for fish transported with and without the addition of water conditioner across different stages of commercial transport. Data are mean (diamond), median, upper and lower 25th percentile, and outliers, $n = 11$. Asterisk indicates a significant difference between treatments within a specific time point (post-hoc Tukey, $p < .05$). Letters indicate differences between specific time points where boxes sharing a letter are not significantly different (post-hoc Tukey, $p < .05$).

[58] J. Balamurugan *et al*, "Clove extract: A potential source for stress free transport of fish," *Aquaculture,* vol. 454, pp. 171–175, 2016. Available: https://www.sciencedirect.com/science/article/pii/S004484861530291X. DOI: 10.1016/j.aquaculture.2015.12.020.

Behavioral response to anesthetic agent in *A. sebae* is markedly similar to that reported for largemouth sea bass, Micropterus salmoides L. (Cooke et al., 2004) with the observation of six induction stages. A rapid anesthetic induction time of less than 180s and recovery time not exceeding 300s are considered to be ideal for fish (Marking and Meyer, 1985). Quicker induction anesthesia in *H. kuda* with clove oil may be due to its high lipophilic nature, which allows it to adhere easily and penetrate the gill epithelium for adsorption in tissues (Mylonas et al., 2005). In clove extract at a concentration of $100\,mgl^{-1}$, the IS6 and RS4 were recorded as 1245±6s and 119±3s, respectively, which could be species specific. Differences in the recovery time also might be related to species, age, size, physiological status, and environmental conditions (Burka et al., 1997, Ross and Ross, 1999). The entry and excretion of anesthetics in fish take place primarily via the gills, whereas the internal organs are only slightly involved (Ross and Ross, 1999) and hence this study on the gill tissue is of prime concern.

The LC_{50} value of clove extract at a concentration of $50\,mgl^{-1}$ observed in this study is relatively similar to the results obtained for sea horse, *H. kuda* with clove oil ($50\,mgl^{-1}$) (Pawar et al., 2011). The other optimized dosages of anesthetic agents such as MS-222 were $75\,mgl^{-1}$ for *Gadus morhua* L. (Mattson and Riple, 1989) and *Solea senegalensis* (Kaup) (Weber et al., 2009), respectively. In this study, it was found that the clove extract is tenfold more potent with regard to induction and recovery time at $100\,mgl^{-1}$ as compared to the clove oil.

The transportation of fish without anesthetics might possibly increase the ammonia concentration through excretion, which affects their gills due to long-term exposure. Several authors have reported about the alterations on the gills of different fish species exposed to ammonia (Mallatt, 1985, Benli et al., 2008). The implication of clove extract on gill tissues was determined in this study by histopathological implications; the optimum dosage of $17.5\,mgl^{-1}$ ensures the prevention of gill damages as compared to control.

[59] 3LittleFish. *Anesthetics / Sedatives*. Available: https://www.3lfish.com/fish-anesthetics-sedatives/. Source for a photo for Section 10.4

[60] U.S. Department of Health and Human Services (FDA), "Concerns related to the use of clove oil as an anesthetic for fish," 2007.

> Historically, clove oil and isoeugenol have been used in foods and eugenol has been used in animal foods. However, concerns regarding this class of chemical compounds led to the nomination of eugenol, isoeugenol, and methyleugenol for investigation under the National Toxicology Program (NTP). The NTP conducts studies in nominated drugs and chemicals to determine their potential to cause cancer. Studies have been completed for eugenol, isoeugenol, and methyleugenol. NTP determined that eugenol is an equivocal carcinogen and methyleugenol is carcinogenic to rodents. While the in-life studies are

[61] A. Gutterman, “Sustainable Product Development,” 2017. Available: https://growthorientedsustainableentrepreneurship.wordpress.com/wp-content/uploads/2017/12/pd_g3-sustainable-product-development.pdf

Distribution stage

- Is transport necessary?
- Volume and nature of transport
- Type of fuel usage
- Eliminate/reduce emissions to air
- Eliminate/reduce waste

Consumption stage

- Eliminate/reduce waste from product
- Eliminate/reduce waste from packaging
- Eliminate/reduce energy consumption

End of life stage

- Extend product life
- Design for repair
- Modular design for maximizing upgradability
- Facilitate recovery of components for reuse
- Facilitate recovery of components for recycling and treatment/disposal

SPSD criteria: Optimize environmental impact

Raw materials stage

- Reduce the volume of materials used (dematerialization)
- Nature of raw materials
- Eliminate or reduce non-renewables usage
- Substitution of none/less hazardous raw materials
- Facilitate recovery, reuse, recycling
- Extraction and processing of raw materials
- Transport from supplier

Production stage

- Optimize production technology
- Eliminate/reduce emissions to air
- Eliminate/reduce effluents
- Eliminate/reduce waste
- Eliminate/reduce energy usage

[62] chillibyte, "How to Transport Your Pet Fish Safely - PBS Pet Travel," *PBS Pet Travel*, Sep. 19, 2014. https://www.pbspettravel.co.uk/blog/how-to-transport-your-pet-fish-safely/

Keep Your Fish in the Dark

Most commonly-kept fish are diurnal, meaning they're awake during the day. At night they typically go into a torpor and are far less active. In this relaxed state they also tend to become less stressed. This can be useful tool. By keeping your fish in a darkened environment (such as placing a sheet or rug over a clear container) can help to minimize the stressful effects of moving your fish.