Live transportation of food fishes: current scenario and future prospects

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Live fish has emerged as a highly in-demand commodity in the recent past due to the progressive quality concepts of seafood consumers. Live fish transportation depends on several internal and external factors which need to be considered critically for improving survival as well as quality during transportation. The lack of a systematic approach for live fish transportation, from on-farm handling to marketing, is the most significant issue faced by the stakeholders. This article provides an integrated insight into the current state of knowledge in the field of live transportation of food fishes emphasizing the significance, present status, challenges and exploration possibilities.

Keywords: Aquaculture, anaesthetization, food fishes, live transportation, water quality.

FOCUSED research on the development of value-added products has been rapidly expanding in the recent past to meet the expectations of globalized consumers in the market. This trend has also impacted the retail marketing of aquatic produce. Market studies have indicated a good appetite for high-end aquatic products with high quality. Live fishes are ideal sources representing top-end value-added products because they guarantee freshness and have higher price realization than fresh, chilled or frozen goods. Marketing live fish attracts consumers for its quality and ensures better revenue for farmers, thereby equalizing demand at both ends. Due to developments in logistics, live fish is occupies a specialized segment in both domestic and international markets. Globally live fish trade is well established mainly in most of Southeast Asia and southern Pacific regions[1]. Initially, in India, the domestic market for live fish transportation was confined to the states in the North East states, but the rising demand for these categories paved the way for a promising market throughout the country. However, meeting the demand for the same was challenging and possible only with a few species of carps as well as air-breathing hardy fishes like cat fishes[2]. Live seafood transportation protocols extend from the use of primitive traditional techniques to huge insulated or non-insulated containers for short-distance transportation. For distant trade, sophisticated hauling trucks with filtration, aeration, refrigeration and water-reticulation facilities employing external sources are used[1]. Regardless of the market potential, the challenges like stress and associated decrease in the survival rate of fishes during transportation limit the exploration possibilities[2–4].

There are several internal as well as external factors which need to be considered critically for improving survival as well as the quality of fishes during their transportation. Density of fish transported as well as water quality parameters like water temperature, pH, dissolved oxygen, carbon dioxide, ammonia, etc. are the major determinants[5]. Appropriate designing of containers for transportation and adherence to standardized operational procedures in every stage is critical for achieving better survival and quality in live fish transportation. This article provides an overview of the live transportation of food fishes and the factors involved. There are a few comprehensive reviews available in this area; hence this article offers updated information for future research in the field of live fish transportation.

Live fish transportation systems

Generally, there are three basic transport systems for live fish trade, viz. closed system, open or tank method, and modified waterless system[6]. A closed system is a sealed unit with all the live transportation requirements (Figure 1), whereas the open system comprises water-filled containers in which transportation facilities are supplied from the outside[7] (Figure 2). The modified waterless transportation system is operated without water except for damping and pre-chilling of transportation medium, viz. sawdust, cotton, etc. for maintaining cool and moist conditions[8] (Figure 3). Various studies have been carried out and patented in the field of live fish transportation, including systems for transportation[9–13] and methods of live fish transportation[14]. Container designing for live fish transportation should consider the quantity and quality of fish to be transported, avoiding sharp projections and abrasive internal surfaces[15].

The open transportation system is ideal for short distances and time duration no longer than 2 h. During transport, air or oxygen should be supplied constantly or intermittently, and water replacement should be followed at regular

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intervals of 4–5 h or less to avoid the warming of water[16]. However, this method has the risk of mortality due to splashing of water, and the approach is often constrained by distance and time. The Australian seafood industry employs three live transportation systems, including polystyrene containers, pickle barrel system and big box system[6]. The first system, which is popular, employs a standard polystyrene box with an inner plastic bag and foam liners. The second system utilizes screw-top plastic containers, whereas the third employs larger boxes with oxygen cylinders strapped on top or inside the box lid. Zhang and Lv[17] refined a refrigeration and oxygenation measurement and control device for live fish transportation. The mechanism also had an independent diesel engine driving power that was independent of the vehicle's regulation, and completely free from the impact of traffic and a variety of emergencies, sustaining the living conditions for the fish. A mobile platform based on the requirements related to behaviour, security, availability, operation and remote control of the system was proposed by Espinosa-Curiel *et al.*[18]. It aided in real-time monitoring of the water quality during transportation of live tilapia fingerlings for increasing the survival rate and reducing stress during transportation. Rifat *et al.*[19] fabricated a simple and energy-efficient aerator-cum-oxygen accumulator for live fish transport using electric power from the vehicle used for transportation. An aerator was constructed using a bilge pump (DC) having a capacity of 1100 GPH operated under 12 V and 3 Å. The equipment was designed to utilize power from the vehicle dynamo while the vehicle was under operation, whereas under static conditions, the power supply unit switched to the battery of the vehicle. Table 1 presents a review of recommendations for the live transportation of food fishes.

The present technological advancements in these strategies demand water, likely increasing the energy consumption and transportation costs[20]. An alternative approach in live fish transportation for increasing biomass and survival rate is waterless transport. Wang *et al.*[21] evaluated the survivability, physiological responses and flesh quality of Amur sturgeon (*Acipenser schrenckii*) during waterless transport. In their study, cold anesthetized fishes were packed into plastic bags filled with pure oxygen, sealed and placed on the trays line by line, which were further piled up in a refrigerated carriage and the temperature maintained around 4°C. They also suggested packing to be done with minimum stress and injury to the subjects and temperature to be maintained below 15°C (ref. 21).

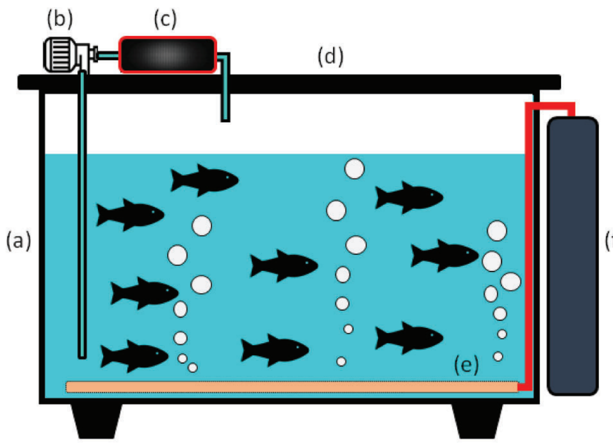

Figure 1. Schematic representation of closed system for live fish transportation. a, Container; b, pump; c, filtration unit; d, lid; e, oxygen diffuser; f, in-built oxygen source.

Factors influencing live fish transportation and associated stress

The survival and quality of live transported fish are the major concerns which depend on various factors. These factors affect the pattern of stress response of transported fish and play a vital role in their overall survival as well as their quality (Figure 4). Stress in fish manifests as a series of complex physical and physiological changes, which ultimately worsens their health status. Failure to reduce fish stress during transportation leads to increased incidence of disease and decreased survival[7]. Stress in fish during live

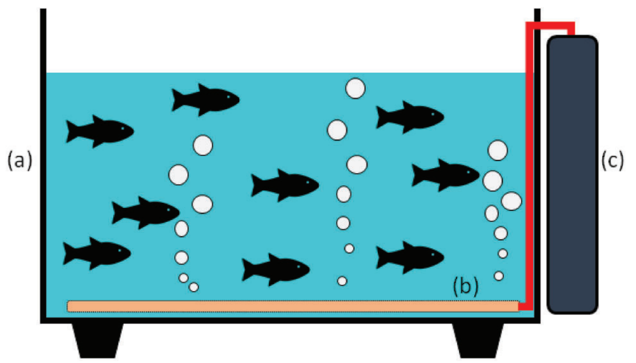

Figure 2. Schematic representation of open system for live fish transportation. a, Container; b, oxygen diffuser; c, external oxygen source.

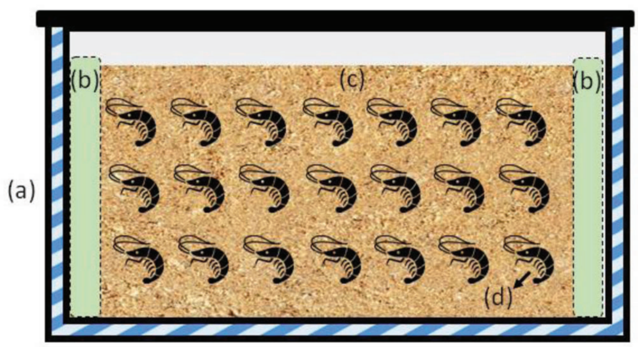

Figure 3. Schematic representation of waterless system for live fish transportation. a, Insulated container with lid; b, cooling unit; c, prechilled layering medium; d, live fish/shellfish.

Table 1. Recommendations for live transportation of food fishes

Species	Pre-transportation	Transportation containers	Transport water	Transportation density	Post transportation	Reference
Fresh water aquaculture species in Bangladesh	Purging for two days. Minimum conditioning period: 12 h. Minimum dissolved oxygen level: 4 ppm Stocking density: 17.7 kg m^{-3}	Plastic tanks of 1 m^3 capacity; aeration by using 4–6 HP diesel pump/oxygen cylinder/battery-operated agitators or compressors	Temperature: 25°C Dissolved oxygen: 6 ppm	200 kg m^{-3}	Partial exchange of water in the transportation tank with that in the stocking tank in order to balance the physico-chemical quality of the water	52
Tiger grouper (*Epinephelus fuscoguttatus*)	Cold anaesthetization from room temperature to 15°C at the rate of 2°C/h	Plastic bags filled with oxygen and water	Temperature: 15°C Ascorbic acid: 25 ppm β-1,3-Glucan: 3.2 ppm	1000 g l^{-1}	–	60
Atlantic salmon (*Salmo salar*)	No purging	40 m live haul vessel having two identical live-hold amidships of 325 m^3 capacity	–	95.5 kg m^{-3}	–	61
Olive flounder (*Paralichthys olivaceus*)	Purging for one day	Flow-through tank	Natural conditions of temperature and photoperiod	16.5 kg m^{-3}	Post-transportation duration: 24 hours	62
Blue tilapia (*Oreochromis Aureus*)	Acclimatized in closed fiberglass tanks with re-circulated water for two days	Plastic bags filled with oxygen and water	Clove oil: 0.2 ppm	80 g l^{-1}	–	63
Silver catfish (*Rhamdia quelen*)	Purging was avoided to simulate market practice	Plastic bags filled with oxygen and water	Eugenol at the rate of 3.0 µl l^{-1}	169.2 g l^{-1}	–	64
European eel (*Anguilla anguilla*)	Purging for four days	Transported in polystyrene transport tank (0.95 × 1.05 × 1 m) filled with 150 l water with recirculation system and supply of pure oxygen	–	270–290 kg m^{-3}	Post-transport stocking density: 72 kg m^{-3} Not fed during the recovery period to avoid adverse water conditions	65
Grass carp (*Ctenopharyngodon idella*)	Acclimatized and purged for one week with a continuous flow of aerated water	–	Eugenol: 10 ppm		–	66
Nile tilapia (*Oreochromis niloticus*)	Purging for one day	Fiberglass box of 1 m^3 capacity, equipped with diffusers and oxygen cylinder	Sodium chloride: 6 ppt	400 kg m^{-3}	–	67
Black rockfish (*Sebastes schlegeli*)	Purging for three days	Transportation unit consists of seawater circulation pump, ultraviolet sterilizer, oxygen generator, protein skimmer and seawater cooling device. Illumination intensity: 60 lux	Temperature: 8°C	198 kg m^{-3}	–	68
Orange-spotted grouper (*Epinephelus coioides*)	Purging for 3 days	Plastic bags filled with oxygen and water	*Glycine tomentella* extract: 250 ppm	200 g l^{-1}	–	69

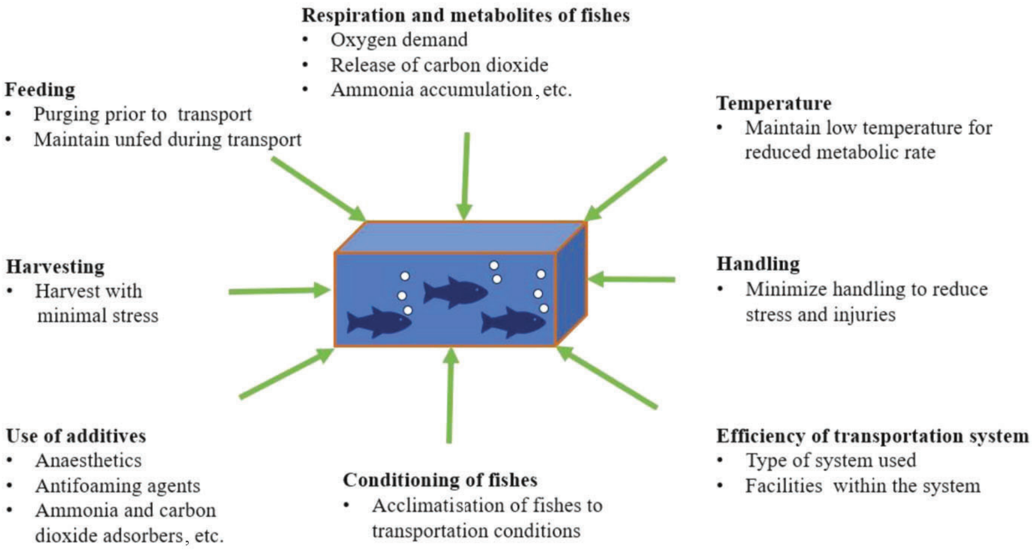

Figure 4. Factors affecting live fish transportation.

transportation is attributed to factors like purging, harvesting methods, handling, water quality parameters such as dissolved oxygen levels, increase in carbon dioxide, drop in pH, increase in ammonia levels in the transportation system, biomass in the transportation system, transportation time, etc.[22]. The resistance to physiological changes creates stressors to pile up, which leads to significant quality changes. Eventually, it leads to a decrease in market value as the ultimate market strategy is to provide healthy fish that survive until they are sold and processed, or re-stocked[23]. Therefore, exporters are cautious not only in mitigating the loss of products due to mortality but also in minimizing the product quality deterioration on account of shipping stress[24]. Stress includes evident physical symptoms like colour variations[25], speedy respiration[26] and behavioural changes[27], while delicate, invisible effects include variations in the fish blood which drastically reduces its capacity to withstand variations in water quality[28]. Countering pH reduction could be the prime factor during short transportation of less than 8 h duration, whereas in long transportation involving more than 8 h, ammonia accumulation is a major concern[29]. Plasma cortisol is the most often used stress marker; it is acutely elevated during brief episodes of transportation but remains high even during long-transport cases[30]. Potential stress factors for live fishes in waterless transportation are ambient temperature, ambient relative humidity, and levels of oxygen and carbon oxide[31]. Indication of the primary stress response of juvenile cod due to handling events during transportation was analysed by invasive measurements of free cortisol release into the tank water[4]. Wang *et al*.[21] reported blood glucose and serum cortisol as reliable markers of stress; alanine aminotransferase and lactic dehydrogenase were the significant markers that reflected the extent of physiological effects on fish caused by stress responses. Anaesthetics were found useful for calming excitable fishes, thereby reducing injuries, whereas they could also bring forth a biological response similar to that caused by stress[32]. Adding sodium chloride (NaCl) to live transport tanks was found helpful to minimize the effects of transport stress. Biswal *et al*.[33] evaluated the stress-alleviating effect of NaCl during simulated live transportation of *Labeo rohita* fingerlings using various biomarkers. They also suggested the addition of 0.4% NaCl in the water for mitigating long-term transformational stress and reducing mortality. According to some studies, enzymes such as serum glutamic pyruvic transaminase[34], lactate dehydrogenase[8], malate dehydrogenase[35] and G-6 phosphatase[36], the stress hormone cortisol[37], metabolites like triglyceride[38] and creatinine[34], and expression of HSP70 mRNA[39] can be successfully deployed as biomarkers to evaluate the degree of transportation stress.

Pre-transportation conditioning

Effective live fish marketing relies to a great extent on the status of the fish prior to transportation. The operational protocol used when transporting live fish includes handling and treatment given to the fish prior to transportation. The quality of the fish will have an impact on its transportation stress and related survival. Fish must be healthy and have sound physical conditions, as excessive mortalities may occur during or post-transportation. Weakened fish are less competent to survive stress during transportation[22]. Studies also recommend the need of information regarding the health status of fish prior to transportation in order to adopt mitigation measures, including lowering of fish biomass being transported, adoption of less stressful harvesting, avoidance of sick and injured fish and careful handling[5,7].

Right from the harvest, responsible capture and transfer strategies with minimal or no injuries and stress should be adopted for effective transportation protocols[40]. Certain recommendations to minimize the effect of transport on fish welfare have been laid out by the World Organization for Animal Health[41], which describes the individual duties and responsibilities involved in fish handling throughout transportation. It is recommended that harvesting be done early in the morning or at night when the ambient temperature is low so as to avoid drastic temperature variations[42]. Less stressful harvesting techniques like line fishing, trapping or harvesting using knotless meshed nets are the best for fin fishes. Further, the basic prerequisite for a stress-free environment is ensuring sufficient clean and oxygenated water for the harvested fishes[41]. The captured fish in highly stressed conditions will have elevated respiration rates and may cause secretions like mucus as well as excreta, which in turn affect the water quality of the holding tank. Studies have shown that keeping fish under starvation, referred to as purging, for one to two days would be advantageous to achieve better survival rates[21,42]. During starvation, the gastrointestinal tract is completely emptied which prevents excess secretion of faeces, reducing the bacterial decomposition as well as ammonia accumulation, and reduces the oxygen requirement ensuring acceptable water quality during transport[22]. Further, fish with empty stomachs are hardier as the energy spent for digesting the food can be used to adapt to a stressful environment.

Anaesthetization

The prime objective of live fish transportation is to supply them to the destination with the maximum possible survival, which can be realized by maintaining the fish under minimal stress. This can be achieved to a great extent by reducing the metabolic rate of the fish during transportation. Extended transportation at a low mortality rate can be achieved by lowering respiration as well as metabolic rate through anaesthetization[43]. There are several anaesthetization techniques such as the use of anaesthetics, application of low temperature and carbon dioxide. Anaesthetics can be categorized into natural and synthetic. The synthetic agents employed for anaesthetization include tricaine methane sulfonate (MS-222)[44], phenoxy ethanol[45] and etomidate[46]. However, accumulation of residues in the meat and adoption of a withdrawal period before harvesting limit the usage of synthetic anaesthetics[47]. Various plant-based natural compounds such as eugenol, menthol, globulol, linalool, guaiol, dehydrofukinone, cineole, spathulenol, caryophyllene oxide, carvacrol, thymol and myrcene have been reported to have anesthetization effects on fishes[47]. However, most of these natural compounds are not listed under GRAS (generally recognized as safe) and can be lethal to fish as well as cause risk to consumers, limiting their application. Temperature-induced cold anaesthetization can be considered as an alternative to the use of anaesthetics for live transport technologies[48]. The fundamental theory underlying temperature-induced anaesthetization is reducing the temperature of the water to a bearable minimum, thus tranquilizing or immobilizing the fishes[49]. Lowering the temperature during transport sedates the fish, lowers their metabolic rate and increases the oxygen saturation level[42]. Generally, temperature reduction is used to sedate the fish rather than absolute anaesthetization, wherein the subjects exhibit weak opercular movements and retain equilibrium to a great extent[49].

Water quality

During live fish transportation, the variations in water quality parameters are complex and inter-related. An increase in fish density thereof can exacerbate water quality issues. Water quality in closed fish transportation systems is a function of the loading density and transportation duration. The main changes in water quality during live transport are low dissolved oxygen levels, increased carbon dioxide, drop in pH and increased ammonia levels. These changes are identified as potential limiting factors owing to excessive respiration, carbon dioxide, and ammonia excretion by the transported fishes[3]. A time-series experiment showed that most of the water-quality degradation occurs rapidly within the first hour after packing. Fishes are prone to chronic stress when exposed to poor water quality, improper stocking densities and inadequate diets for a prolonged period[41]. Water temperature is a critical parameter during live fish transportation as it influences and induces rapid variations in other quality parameters such as pH, dissolved oxygen, carbon dioxide, ammonia, etc. The rate of water exchange and stocking density are the determining factors that affect water quality in open systems, whereas a decrease in pH, accumulation of toxic metabolites such as carbon dioxide, ammonia, and organic carbon leads to the deterioration of water quality in closed systems of live fish transportation[50]. Studies have indicated that the oxygen-carrying capacity of transporting water could be increased by lowering the water temperature[50]. Svobodova *et al.*[51] recommended minimum dissolved oxygen requirement as 6 ppm (70% saturation) for cold-water fish, 5 ppm (80% saturation) for tropical freshwater fish and 5 ppm (75% saturation) for tropical marine fish. During live fish transportation, dissolved oxygen should be maintained around 100% saturation or carbon dioxide level should be kept below 20 ppm. The toxicity of ammonia indicates direct relation with pH and reported lethality even at a low level (2 ppm), which could be minimized by fasting fish prior to transport and/or adding ammonia-reducing agents to the transport water[52]. During simulated live transportation of cod (*Gadus morhua*)[4], a marked drop in pH within an hour was observed, which further lowered below 7 after 6 h of transportation. Buffers such as TRIS, sodium and bicarbonate were found advantageous for maintaining pH during live fish transportation. The acceptable pH for fish normally ranged between 7.5 and 8.5 and was recommended to maintain total alkalinity values above 50 ppm (ref. 53). Low pH results in physiological problems primarily affecting the oxygen-utilizing ability leading to respiratory stress even when dissolved oxygen levels are high. This declining pH in fish transport applications directly affects carbon dioxide accumulation[54]. A variety of chemical packs that produce oxygen[55] as well as ammonia/carbon dioxide scavengers for live fish transport applications are employed, viz. immersion packs which are placed indirectly outside the inner bags for release of oxygen in the transportation system[56]. Prototype live fish transport systems using sodalime effectively reduced carbon dioxide levels and increased pH in the transport medium[6].

Revitalization process and post-transportation survival

Transferring transported fish to holding tanks at their destination is a simple process, but it must be done carefully to avoid fish mortality. The water quality in transport bags will be considerably different from that in the holding tank, and the fish should be transferred with extreme caution[57]. The survival rate associated with the transported fish is highly influenced by the conditions used during revitalization. Immediate aeration of the transported environment is necessary to remove accumulated carbon dioxide and clean saltwater equal to the volume in the transported box was added to acclimatize fish to holding system conditions[6]. Increased survival rates were obtained for fishes when allowed to recover in enriched saline water after live transport[58]. The addition of sodium chloride in the transport water was found to be advantageous improving survival rates.

Status and prospects of live fish trade

Live fish trade has increased in the recent past on account of the lucrative market for these commodities. Live, fresh or chilled fishes represent the largest market share (44%) utilized for direct human consumption, often being the most preferred and highly priced commodity[59]. Southeast Asia is the dominant market for live fish and Hong Kong is considered the centre of live food fish trade. A well-established supply chain from fishermen to end-consumers has been developed in the coastal waters of Southeast Asia. In the Indian context, the live fish products are sold on a modest scale to both domestic and international markets. This generates additional revenue for aqua farmers and considerable foreign exchange for India by opening up new markets for seafood exports. The country has exported 7287 MT of live fish worth Rs 324.26 crores during 2019–20, which is a high price realization compared to the other processed commodities like chilled forms[8]. Currently, the major live items exported from India are mainly crustaceans, including mud crabs, blood clams, horn shells, murex, lobsters, etc. with the major export hub of India being Chennai and the market being Southeast Asian countries. However, a few challenges associated with this live export trade include higher air-freight charges, delay or logistic interruptions resulting in higher mortality, bulk and cost of live transportation systems and the lack of a well-established supply chain. Even though a few live transportation systems are commercially available, most stakeholders are unwilling to adopt such technologies as they are unaffordable. Considering the practical feasibility, simple transportation systems like styrofoam boxes are used as alternatives, especially for waterless transportation but they pose adverse environmental consequences.

Conclusion

In the present scenario, the live fish trade requires a smart live fish transportation system self-capable of manipulating the transport conditions to accommodate a wide range of live species. The various water additives such as antifoaming agents, NH_3 and CO_2 absorbers, other herbal and synthetic aesthetics for mitigating potential stressors during live seafood trade are underutilized on a commercial basis. Despite the widespread application of food traceability systems for various types of seafood, its adoption as well as the standard operating procedures (SOPs) to be followed in live fish transportation must be fully developed. A traceability platform combined with species-specific SOPs would provide optimal micro-environmental conditions for the target species resulting in improved survival and quality while reducing transportation risks. The introduction of real-time quality control mechanisms can dynamically guarantee the survival rate and food quality after developing species-specific optimized protocols. Hence, focused research and development on these aspects can help develop an effective live fish transportation device with due consideration of its economic feasibility, to meet the needs of stakeholders across the seafood value chain.

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