The current state of biosecurity and welfare of ornamental fish population in pet fish stores in Chiang Mai Province, Thailand

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Abstract

Aquaculture has undergone extensive development in recent decades due to its use as a source of protein for human consumption, as models for scientific research, and as pets. The purpose of this study was to survey ornamental fish stores in Chiang Mai province, Thailand to identify practices which affect life quality and welfare of pet fish including general management, biosecurity management, and knowledge about fish disease and drug usage of ornamental fish store owners. The results show that most pet fish stores have poor husbandry in terms of fish health based on the observed incidence of skin erosion and fin rot (92.86%) and white spots on the skin (78.57%) in store fish. Moreover, treatment of these health conditions were performed by experienced people working in the stores without consulting a veterinarian. The top three drugs used for treatment included malachite green oxalate, trichlorfon, and formalin. Interestingly, oxytetracycline and chlortetracycline were also used frequently to treat fish disease. Despite a lack of formal training in fish management, fish health, and drug usage in ornamental fish, the knowledge of owners about fish disease and antibacterial agent usage was determined to be of an intermediate level based on testing. The information gained from this study can be used in future studies to identify stressors that affect pet fish welfare and to investigate biosecurity and ornamental fish welfare in the other sectors of the ornamental fish trade supply chain.

Keywords: Biosecurity management, Chiang Mai, Fish health, Fish welfare, Pet fish stores

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INTRODUCTION

Over one billion fish are traded globally each year. A shift in the demand for fish promoted a sharp increase in recent years (Qi, 2002; Goessling and Sadler, 2015). Fish have essential roles and are increasingly being used in aquaculture, pet keeping, and in scientific research (Qi, 2002; Goessling and Sadler, 2015). Asia is the major supplier of fish, with Thailand being one of the leading producers of fish (Choo and Williams, 2003). However, there are many negative impacts of fish production and these problems are difficult to assess adequately (Tavares et al., 2017). Serious problems include a lack of good management and fish disease outbreaks from bacteria (Oladele et al., 2015; Sahoo et al., 2016) viruses (Pikulkaew et al., 2009; Pikulkaew et al., 2020), and parasites (Leung and Bates, 2013; Videira et al., 2016) which have been identified as a public health problem due to antimicrobial-resistance (Watts et al., 2017; Saengsitthisak et al., 2020).

Fish supply chain practices are a part of the sustainable supply chain management that has gained greater importance in the last 25 years, as issues have been identified at various levels of organization. Of particular concern is the quality of products that consumers demand and their satisfaction (Malindretos et al., 2016). The fish trade supply chain commonly involves growers, distributors, middlemen, wholesale exporters and importers, retailers in local stores, and consumers. Each plays an important role in the animal distribution process and fish health and welfare are affected by each step of the supply chain (Islam and Habib, 2013). Thus, not only the action of growers, including fish management in the supply chain have important effects on fishery production, but retailers in local stores also play an important part. The Thai agriculture government has documented good aquaculture practices for ornamental freshwater farmers (ACFS, 2020). Huntinford and Kadri (2014) reported that while farmed fish welfare is frequently accessed and promoted, good management practices and welfare in the pet fish supply chain were lower priorities (Huntinford and Kadri, 2014; Magada and Mercy, 2016).

Although the high value obtained from the animal-production sector is realized, the welfare of animals also needs to be recognized (Bovenkerk and Meijboom, 2013; Sánchez-Suárez et al., 2020). Nowadays, welfare is more highly regarded not only in mammals but also in non-mammals and fish (Mather, 2019; Sánchez-Suárez et al., 2020). The topic of farmed fish welfare has been subject to increased public and scientific scrutiny. The welfare of fish is deemed good if they can adapt to live in their environment, are able to lead a natural life, and are in good health. Moreover, animals should be free of negative experiences (Bovenkerk and Meijboom, 2013; Mather, 2019; Sánchez-Suárez et al., 2020). Based on new knowledge related to fish physiology and cognitive functions, the general scientific opinion is that fish are capable of experiencing suffering (Huntingford et al., 2006; Pleeging and Moons, 2017). Scientific research has documented that fish are most likely sentient beings, like other animal such as mammals and birds, because they share the same complex behaviors (Pleeging and Moons, 2017). Moreover, the growing knowledge of stress response and the negative impact of chronic stress in fish have led to the conclusion that fish can suffer due to stress (Huntingford et al., 2006; Pleeging and Moons, 2017). One of the primary challenges in welfare studies of ornamental fish are defining the optimum conditions that may provoke undesired physiological responses in different
species which are dependent on each species’ normal habitat and environment which differs from food fish management. The appropriate conditions for pet fish and the tolerances and adaptive capabilities are diverse and vary strongly among fish species, thus there is no one-size-fits-all approach (Stevens et al., 2017).

Base line management information from pet stores that involved or is related to the fish keeping should identify factors related fish health and their welfare. These data can be used to generate further research such as the investigation of the relation between the physical environment and fish health and/or their life quality and welfare, examine potential welfare problems in specific species of ornamental fish to provide species-specific welfare data. The outcome of these studies would contribute to the development and improvement of fish welfare regulations and define standard methods to assess animal welfare that are feasible for use on working sites (Bovenkerk et al., 2013, Pleeging et al., 2017, Stevens et al., 2017).

The objectives of this study are to survey and collect data from pet stores using a questionnaire to obtain information on general management, biosecurity management, and perceptions and knowledge about fish disease and drug usage by owners of ornamental fish stores located in Chiang Mai province, Thailand and also to assessed fish disease and knowledge on antimicrobial agents of them. The results will provide information that will help identify stressors that affect to pet fish welfare and identify welfare issues to investigate in the other sections of the supply chain trade.

MATERIALS and METHODS

Survey population

Our target population was ornamental fish stores in Chiang Mai, Thailand. The sample consisted of all registered and active ornamental fish stores in Chiang Mai, Thailand in 2018 (n = 18). The survey was conducted to determine the number of ornamental fish stores in Chiang Mai, Thailand based on the database of the Chiang Mai Fisheries Government accessed on 10 June 2018 (Available from: https://www.fisheries.go.th/fpo-chiangmai/web2/), moreover Global Positioning System (GPS) was used to specify their locations of each fish pet fish stores and Quantum Information System (QGIS) version 2.18.28 was used to display location information in form of maps. The survey covered events from August 2018 to July 2019.

Before filling the questionnaire, the aim and research methodology of the study were explained to participants who gave informed consent prior to completing the questionnaire survey and were assured that participation was voluntary and results would be anonymous. The face-to-face interviews and information disclosed were recorded by the researcher. Only persons involved to the research had access to the result. In addition, we concerned to human ethic requirement, the procedure this study was followed policy and requirement of university which realized base on The Belmont Report that are respect for persons, beneficence and justice. No animal samples were collected in this study. Geographical coordinates (X–Y coordinates) that were described the X value in term of longitude and the Y value in term of latitude, were recorded when visiting the stores and tank and fish inventory data recorded.
Questionnaire design  
The questionnaire consisted of 45 items and was designed to gather general information and specific data on general management and biosecurity management in ornamental fish stores. Moreover, information about knowledge of fish diseases and the drug use of the owners of the stores were collected using self-assessments and a quiz. The survey questionnaire was developed, accessed, and validated for use in this study.

Data collection and data analysis

Data collection
The questionnaire was designed to include open and closed questions, and both categorical and numerical data were collected. The questionnaire was divided in 3 parts with different topics including general information including the source of ornamental fish, general management, and biosecurity management in ornamental fish stores.

The first part consisted of questions to collect information about the stores, such as the name of the owner and name of the store, the location of the store, and the duration of business. The survey questionnaire collected data for assessment in the second and third parts.

Data processing
Information from the interviews was recorded, organized, and summarized using Microsoft Office Excel spreadsheets (www.microsoft.com). Data were exported for analysis into R (Version 3.42) (R Core Team, 2019; Stevenson et al., 2019).

Statistical analysis
Data were analyzed using descriptive statistics, including frequencies and percentages. Moreover, a relationship between the knowledge level of the shopkeepers and mortality percentage of fish disease was determined by Fisher’s exact test. Descriptive statistical tests were performed using R (Version 3.42). Moreover, heat map analysis by R (Version 3.42) to illustrate relation between fish stores and their destination for fish trades (R core Team, 2019; Stevenson et al., 2019).

RESULTS

Survey population
The database of the Chiang Mai Fisheries Government that was accessed on 10 June 2018 and 18 ornamental fish stores in Chiang Mai were identified for the survey (Department of fisheries, 2018). The locations of the ornamental fish stores are shown in Figure 1. Fifteen stores were located in Mueang, Chiang Mai and the remaining stores that were 2 stores located in San-Sai and 1 store located in Saraphee. Four owners declined to participate in the questionnaire but allowed tank and fish inventory data to be collected for the trial. In addition, 12 (66.67%) of the interviewed ornamental fish stores were registered and had been active for more than 3 years. In addition, 2 (11.11%) of the stores had operated for less than 1 year. All stores were wholesalers and retailers, but 4 stores also breed fish.
Descriptive statistics from the questionnaire survey

General management

Using visual observation, we documented the number of tanks and fish in each of the 18 stores. The stores were classified into 3 groups: less than 50 tanks (9 stores; 50.00%), 51–100 tanks, (7 stores; 38.89%), and more than 100 tanks (2 stores; 11.11%) (Table 1). Ground water was found to be the major water source for the stores; however, 7 (50.00%) of the 14 interviewed stores filtered and/or treated water before filling the tanks. All stores used a water circulation system for individual tanks. This action was performed because in each store, cross-contamination of pathogens from difference sources was considered.
The major of ornamental fish farms, which are part of the fish trade supply chain in Thailand, are in the surrounding provinces of the central region including Nakhon Pathom, Pathum Thani, Ratchaburi and Bangkok. The biggest production area is Ratchaburi, which is famous for ornamental fish farms. Moreover, Japan and Indonesia are the source of the obtained fish for the pet stores (Figure 2). From observations made in the store and interviews of store owners, it was found that all 18 stores sold freshwater ornamental fish. The various types of ornamental fish are summarized in Table 2. The top three best sellers were Goldfish (Carassius auratus Linnaeus, 1758), Koi carp (Cyprinus carpio Linnaeus, 1758), and red swordtails (Xiphophorus hellerii Heckel, 1848). However, interviews of the owners indicated that the quantity of Goldfish (Carassius auratus Linnaeus, 1758) decreased in the winter because these fish easily became sick and have a high mortality rate. In addition, during the rainy season sales of Guppies (Poecilia reticulata Peters, 1859) increase.

**Table 1** Information of the general management and biosecurity management.

| Topics in questionnaire               | N (%)                        |
|--------------------------------------|------------------------------|
| 1 Amount of tanks in store           |                              |
| • < 50 tanks                         | 9 (50.00%)                   |
| • 51-100 tanks                       | 7 (38.89%)                   |
| • > 100 tanks                        | 2 (11.11%)                   |
| 2 Sources of water                   |                              |
| • Tap supply                         | 5 (35.71%)                   |
| • Ground water                       | 9 (64.29%)                   |
| 3 Circulation of water system        |                              |
| • Separate system                    | 14 (100.00%)                 |
| • Circulate system                   | 0 (0.00%)                    |
| • Combination                        | 0 (0.00%)                    |
| 4 Treat/filter water before used     |                              |
| • Yes                                | 7 (50.00%)                   |
| • No                                 | 7 (50.00%)                   |
| 5 Number of ornamental fish in store |                              |
| • 1000-5000                          | 9 (64.29%)                   |
| • 5001-10000                         | 3 (21.43%)                   |
| • > 10000                            | 2 (14.29%)                   |
| 6 Frequency of order ornamental fish |                              |
| • 1 time per month                   | 2 (14.25%)                   |
| • 2 times per month                  | 3 (21.43%)                   |
| • More than 3 times per month        | 9 (64.29%)                   |
**Table 2** Types of ornamental fish distributed in stores located in Chiang Mai.

| Order* | Scientific name* |
|--------|------------------|
| Cyprinodontiformes | Guppy (*Poecilia reticulata* Peters, 1859)  
Red swordtails (*Xiphophorus hellerii* Heckel, 1848) |
| Perciformes | Siamese fighting fish (*Betta splendens* Regan, 1910)  
Freshwater angelfish (*Pterophyllum scalare* Schultze, 1823) |
| Cypriniformes | Zebrafish (*Danio rerio* Hamilton, 1822)  
Goldfish (*Carassius auratus* Linnaeus, 1758)  
Koi carp (*Cyprinus carpio* Linnaeus, 1758) |
| Characiformes | Neon tetra (*Paracheirodon innesi* Myers, 1936) |
| Cichliformes | Blood parrot cichlid (*Amphilophus citrinellus × Cichlasoma synspilum*) |

* Information of taxonomy and scientific name based on https://www.fishbase.se/search.php [Available online: 15 December 2020].

**Figure 2** Heat map represents patterns between fish stores and their destination for fish trades. Columns represent ornamental stores code which were interviewed and rows represent the destination of the ornamental fish. Orange blocks indicate that provinces or countries (red letters) are the source of the obtained fish for that pet stores.
Biosecurity management

Animal management

Ten (71.43%) stores stated that they feed their fish once daily because they do not want their fish to defecate when sold, as this pollutes the water. The amount of feed offered depended on the size of the tank and the quantity and density of fish, which was based on experience of the owner. Any unconsumed feed was removed to prevent wastewater production.

In all 14 stores, ornamental fish were quarantined and separated to prevent cross-contamination before being stored in stores. Each store reported having more than one source of ornamental fish. The main sources of fish were from the central region of Thailand, especially Ratchaburi, that is the major pet fish breeding or farming are within the country. The imported ornamental fish were mostly obtained from Japan. Moreover, the stores separate fish by size, type, sex (in some cases), and condition with consideration for the density of fish in each tank before selling. An incidence rate of 1–2 sick fish per month was reported for nine (64.29%) stores. Thirteen (92.86%) stores stated that they separate and quarantine sick fish before treating them. Moreover, we observed that only 5 (35.71%) stores consulted a veterinarian when a fish dies.

Management of diseased fish in each store involved various methods to treat and prevent cross infection to other fish depending on the condition. However, the same key procedures were used by all owners that was the first step was assessment of whether the disease is transmissible or not. Then, if it was considered a transmissible disease, all fish in that tank were treated and a sign telling customers that the fish in the tank are not for sale until the fish recover is displayed. In cases of dead fish, differences were reported in the management used by each store. Nine (64.29%) stores put the fish carcasses into a plastic bag that was disposed of using a private or municipal garbage disposal organization. Other stores fed dead fish bodies to other animals or buried the carcasses.

Two types of fish tank cleaning management were identified: 100% cleaning and partial cleaning (20–30% of water removed and replaced with freshwater). Complete cleaning of tanks was performed when all fish in a tank had been sold. However, only 1 store reported that this process occurs when there was a sick fish in the tank. Nine (64.29%) stores stated that they partially clean their tanks 2–3 times per week. Furthermore, all ornamental fish stores stated that they do not treat water before distributing it to the environment. Fish hand net use was reported to be separated between each tank by four (28.57%) stores. However, all 14 stores stated that fish hand nets were cleaned, sterilized, and dried daily after store closure.

Pathogen management

The frequency of observation for fish illness was reported to be 1–2 times/month for 9 stores (64.29%) and every day for 2 stores (14.29%). The signs and symptoms of fish illness that are mostly observed in stores were reported to be skin erosion and fin rot (13 stores; 92.86%), white spots on the skin (11 stores, 78.57%), visible parasite infection (7 stores, 50.00%), and skin hemorrhage (3 stores, 21.43%). The prevalence of each sign and symptom and the mortality rate reported for all stores is summarized in Table 3.
Table 3 Prevalence and mortality rates for different symptoms or diseases in the fish stores.

| Disease/sign symptom          | Report store n (%) | Prevalence | Mortality rate |
|------------------------------|--------------------|------------|----------------|
|                              | %                  | n (%)      | %              | n (%)          |
|                              | <20%               | 3 (27.27%) | <10%           | 8 (72.73%)     |
|                              | 21–30%             | 6 (54.55%) | 11–20%         | 2 (18.18%)     |
|                              | 30–40%             | 1 (9.09%)  | >20%           | 1 (9.09%)      |
| White spots on skin          | >40%               | 1 (9.09%)  |                |                |
|                              | Found 11 (78.57%)   |            |                |                |
|                              | Not found 3 (21.43%)|            |                |                |
|                              | <5%                | 1 (7.69%)  | <10%           | 3 (23.08%)     |
| Skin erosion and fin rot     | 10–20%             | 8 (61.54%) | 10–20%         | 8 (61.54%)     |
|                              | 20–30%             | 3 (23.08%) | 20–30%         | 1 (7.69%)      |
|                              | 30–40%             | 1 (7.69%)  | >30%           | 1 (7.69%)      |
|                              | Found 13 (92.86%)   |            |                |                |
|                              | Not found 1 (7.14%) |            |                |                |
|                              | 5–10%              | 3 (50.00%) | 0%             | 3 (75.00%)     |
| Visible parasite infection   | 4 (50.00%)         | 5%         | 1 (25.00%)     |
|                              | Not found 7 (50.00%)|            | 10–20%         | 3 (100.00%)    |
|                              | 10%                | 2 (66.66%) | 5–10%          | 2 (66.66%)     |
|                              | 20%                | 1 (33.33%) | 30%            | 1 (33.33%)     |
| Skin hemorrhage              | Found 3 (21.43%)   |            |                |                |
|                              | Not found 1 (78.57%)|            |                |                |

a Percentage of disease/sign prevalence
b Amount of stores (percentage of stores) that reported the prevalence of disease/sign
c Percentage of mortality rate from disease/sign prevalence
d Amount of stores (percentage of stores) that reported the mortality rate from disease/sign prevalence

We investigated whether fish with severe cases of illness were separated for treatment. In cases of non-communicable diseases, fish were moved and separated for treatment. The fish remaining in the tank had their water completely changed and/or were treated. All 14 stores stated that they diagnose and treat fish themselves without consulting a veterinarian. Furthermore, they stated that they make decisions about drug use based on their own experience.

Figure 3-4 summarized the drugs and chemical agents, and antimicrobial agents used to treat and prevent disease in fish in the stores. The top three drugs and chemical agents used in the stores were malachite green oxalate, trichlorfon, and formalin. In addition, nifurstyrenat-sodium 9 (64.29%), diflubenzuron 3 (21.43%), a mixture of praziquantel, diflubenzuron, and metronidazole, and acriflavine 2 (14.29%). Interestingly, 7 antibacterial agents used to treat animals were identified in this study. We also observed that oxytetracycline and chlortetracycline, which are antimicrobials used to treat human infections, were also used to treat fish disease.
**Figure 3** The drugs and chemicals used in the fish stores. A Hazardous substance classification: 1* Trader of this substance has to follow guidelines and laws for manufacture, import, export, or distribution; 2* Trader of this substance has to register for operation for manufacture, import, export, or distribution; 3* Trader of this substance has to register and have a license for manufacture, import, export, or distribution (only ornamental fish treatment allowed); 4* This substance is not allowed to be manufactured, imported, exported, or distributed. (1*–4* were classified by the Department of Fisheries, Thailand; available online: https://www.fisheries.go.th/train-gr/coastal/001/02/Drop_DOF.pdf on date 19-12-2020). NC* This substance is not classified in the category of hazardous substances by the Department of Fisheries, but is used as a drug for humans or other animals.

**Figure 4** Antibacterial agents that are used in stores. This information was recorded through interviews (n = 14 stores).
People management

Owners in 6 of the 14 ornamental fish stores that completed the questionnaire graduated from a formal program and/or had taken individual courses on fishery, ornamental fish, or related subjects that could aid in management of the stores. Moreover, all 14 stores (100%) informed us that they had not obtained information about management of an ornamental fish store, ornamental fish health, or drug and chemical usage from academic and/or a related government organization. Fifty percent of storeowners stated that they carry out drug and chemical treatment themselves. This practice would potentially contribute to increased antimicrobial resistance, which is a public health problem.

In this study, we also examined the owners’ knowledge on antibacterial agents and fish disease though self-assessments and a quiz. The criteria for the exam-assessment knowledge levels were high, intermediate, low, or none based on quiz scores of >75%, 50%, >25%, and <25%, respectively. Most owners assessed their knowledge at an intermediate level. Interestingly, none of the storeowners assessed their knowledge of antimicrobial agents at a high level, but 2 (14.28%) individuals scored high for fish disease knowledge. In regards to antibacterial agent knowledge, 6 (42.83%) scored high and 6 (42.83%) scored intermediate on the exam-based assessment (Table 4).

Table 4 Assessment of the knowledge of ornamental store owners on fish disease and antibacterial agents using self and examination-based assessment methods.

| Knowledge levels | Fish disease | Antibacterial agents |
|------------------|--------------|----------------------|
|                  | Self-assessment | Exam assessment | Self-assessment | Exam assessment |
| High             | 2 (14.28%)     | 3 (21.43%)          | 0 (0.00%)       | 6 (42.83%)       |
| Intermediate     | 12 (85.72%)    | 9 (64.29%)          | 8 (57.14%)      | 6 (42.83%)       |
| Low              | 0 (0.00%)      | 2 (14.28%)          | 3 (21.43%)      | 2 (14.28%)       |
| None             | 0 (0.00%)      | 0 (0.00%)           | 3 (21.43%)      | 0 (0.00%)        |

1Exam-assessment levels were: high = score of more than 75%; intermediate = score of more than 50%; low = score of more than or equal 25%; and none = score of less than 25%.

DISCUSSION

Most people regard fish as food or pets. Fish are the most frequently consumed animal in terms of number and the most common kind of pet. Keeping a pet fish is a hobby associated with relaxation. Fish are also used in scientific research and are second only to mice in terms of number used for research (Brown, 2015; Clements et al., 2019;). Chiang Mai is the largest province and the hub of northern Thailand. It has a population of more than 1 million and covers 20,107.057 km² at a latitude of 18.7903805 and a longitude of 98.9846802. It is located on plains at an elevation of 310 m and is surrounded by the Thai highlands mountain range (Available from: http://www.chiangmai.go.th/mang/public/D8/8D01Feb2017150134.pdf). Even though Chiang Mai is not the major producer and exporter of ornamental fish in Thailand, the demand for ornamental fish in Chiang Mai is high. There are 18 ornamental fish wholesale...
According to the Aquatic Plants and Ornamental Fish Research Institute (APOFRI) Inland Fisheries Research Development Bureau, Department of Fisheries, Thailand, Thailand is one of the major exporters of ornamental fish worldwide. Exports are made to over 50 countries worldwide with a total yearly export value in excess of 30 million US dollars. Germany, UK, Netherlands, France, Italy, USA, Singapore, Japan, and Hong Kong are the main markets for Thai ornamental fish. The major of ornamental fish farms, which are part of the fish trade supply chain in Thailand, are in the surrounding provinces of the central region including Bangkok, Ratchaburi, Nonthaburi, Chachoengsao, Nakhon Pathom, and Suphan Buri. The biggest production area is Ratchaburi, which is famous for breeding koi, guppy, and goldfish. Bangkok is the discus fish center while Nakhon Pathom has the biggest Siamese fighting fish farm as reported in a previous study (Available from: https://www.fisheries.go.th/aquaorna/web2/images/download/institue%20in%20D.pdf). The geographical features of Chiang Mai are not suitable for pet fish farming. Thus, most ornamental fish stores in Chiang Mai act as wholesalers and retailers in the ornamental fish trade supply chain.

Successful pet fish production includes good management that not only considers economic value loss but is also concentrated on the welfare of fish including fish quality of life and health. However, the major challenges within the supply chain regarding caged fish are inappropriate holding conditions, poor water quality, handling, transport, confinement, crowding, poor diet or feeding methods, and disease. These issues can be solved through the application of good practices and procedures combined with attention to fish welfare management. Fish welfare is defined as the fish has good health, they can obtain what they want, and they do not experience negative emotions and/or stress inducers. Moreover, welfare in fishes also include experience pain and suffering, or even experience consciousness. Presently, fish welfare awareness is rising and many globally animal ethic organizations are working to generate methods of assessing fish welfare in all areas the aquaculture. This is especially true for stressors of fish in each segment of the ornamental fish trade. Regrettably, both transportation and handling are stress in fishes that cannot be avoided completely. Handling fish for as little as 30 s has been reported to increase cortisol, plasma glucose, haemoglobin and haematocrit in a number of fish species, as well as increasing the fish’s metabolic rate (Stevens et al., 2017). Hence, reduction of chronic stress is likely to reduce mortality and improve welfare (Stevens et al., 2017).

Health management within the ornamental fish market starts with the concept of prevention, which is accomplished through proper management of water quality, e.g., factors such as the oxygen content, pH, salinity, temperature, nutrition and feeding, and sanitation. All of these factors are also fish welfare indicators, as appropriate conditions will decrease stress in fish. As stated in the proverb “an ounce of prevention is work a pound of cure”, proper ornamental fish management in the industry chain could decrease economic losses and mortality rates, increase production, and reduce the spread of pathogens that
may carry disease and antimicrobial resistance genes to other animals, humans, and/or the environment (Miller Morgan et al., 2012; Huntingford and Kadri, 2014; Gopakumar, 2016; Stevens et al., 2017).

Water quality is the primary environmental consideration regarding the welfare risk assessment of fish and has the potential to markedly affect the health of fish. Sudden changes in the environment, such as changes in the feeding rate or stocking density can elicit rapid changes in the water quality. To reduce waste build-up, the accumulation of metabolic waste in the water should be avoided (Stevens et al., 2017; Yildiz et al., 2017). The pet stores interviewed in this study, which were retail stores (fish were kept in stores for less than 2 weeks), determined the appropriate frequency and quantity of feeding based on preventing poor water quality. Uneaten feed, waste excreted by fish, chemicals, and therapeutics comprise the waste produced. Four stores stated that they feed their fish once each day and/or only in the evening because they wanted to reduce the amount of fish biomass per bag as well as reduce the risk of metabolic waste build-up when fish are sold on the following day. Overfeeding, which means feeding in excess of intake, causes food waste which pollutes the environment. Underfeeding results in reduced growth, increased aggression, and interfish competition for feed, and in the worse cases, fish may die (Attia et al., 2017). Liang et al. (2013) and Kosemani et al. (2017) reported that the feeding frequency and practices affect feed intake of fish, the quantity of uneaten feed, feed efficiency and, consequently, metabolites and excreta and water quality. Talbot et al. (1999) suggested feed management strategies such as the amount of feed fed, frequency of feed delivery (number of meals per day), timing of meals, and the spatial distribution of the feed, as well as the fish type are important management practices for maintain water quality. Thus, appropriate feeding is very important for the health, behavior, and welfare of fish.

Interestingly, the fish in all 14 stores were of various types and species. They were quarantined, separated, and the density of fish was controlled by the owner in each tank. Tanks with mixed species and sizes have to be carefully maintained because different species may have different optimal ranges in terms of the water quality and stocking density of each type of fish. If any of the variables are unsuitable, the fish may survive but not necessarily thrive. There is concern when retailers or consumers place fish into community tanks with other species (Stevens et al., 2017) as the stock density of ornamental fish needs to be determined with social tendencies and tank size in mind. Many species of fish live in large, coordinated social groups or schools, but these conditions are not suitable for some of species of fish (Yildiz et al., 2017). Ramsay et al. (2006) reported that when fish are maintained in crowded conditions, some popular pet and research species have shown elevated cortisol concentrations indicating stress. The results of our study indicate that storeowners use their knowledge and experience to determine the density of fish in each tank rather than any industry standard. The effects of stocking density on the welfare of fish can be measured and/or assessed by the behavior status, physical status, or health status of the fish, which can be observed.

Handling can also cause stress in fish and occurs primarily in the ornamental trade. Handling fish for as little as 30 seconds could cause a strong
stress response, and this has been reported to increase following capture or for husbandry reasons when removing them from water (Falakhtar et al., 2009; Stevens et al., 2017). Our study found that all stores use nets to handle fish, and some of stores use separate nets for each tank. To reduce stress, there are many techniques, including the use of nets that are designed to prevent the disruption of scales or the mucous layer on the skin surface. Moreover, nets can protect fish and reduce bumping to other fish when lifting fish from the water (Stevens et al., 2017). Because of our study, we identified poor animal husbandry in fish production and pet keeping so the occurrence of sick fish and/or chronic fish diseases cannot be avoided. Chronically or repeatedly stressed fish experience immunosuppression and, consequently, are at greater risk of disease. Many ornamental fish diseases are caused by pathogens that are natural inhabitants in water and tend to manifest opportunistically in times of stress (e.g., white spot disease, caused by the protozoan Ichthyophthirius multifiliis) (Stevens et al., 2017; Noga, 2010). The occurrence of disease in stressed fish has been reported in both aquaculture and ornamental fish (Ramsay et al., 2010; Sung et al., 2011). Thirteen (92.86%) stores stated that they had experienced skin erosion and rot and 11 (78.57%) had experience white spot disease in our study. However, infected fish in 13 (92.86%) stores were separated from healthy ones when they got sick. This management practice has been reported to inflict less stress on fish (Assefa and Abunna, 2018).

The information obtained from interviews revealed that most preventative measures and treatment of fish in stores was based on their appearance, i.e., signs and symptoms of disease, without consulting a veterinarian or conducting a laboratory examination. In addition, stores concentrated on disease prevention practices, treatments, and decisions using drugs, chemical agents, and antibacterial agents based on the owners’ experience, even though owners of 8 (57.14%) of 14 stores had not graduated and/or completed training in a course about fishery or related topics. No stores had obtained information about retail or wholesale fish management, ornamental fish health, or drug and chemical usage from academic and/or related government organizations. Without the appropriate choice and application of treatment, excess stress will be inflicted on fish (Midtlyng, 1997; Stevens et al., 2017). Hence, the drugs, chemical agents, and especially antibacterial agents were applied without appropriate discrimination that could cause serious public health problems such as multidrug-resistance. Antibiotic resistant genes have been reported in ornamental fish sampled in the UK (Verner-jeffreys et al., 2009). Interestingly, malachite green, which was found in most of the shops surveyed, was reported to have carcinogenic effects that could affect people who have direct contact with this substance or indirectly by distribution of the contaminated water into the environment (Culp and Beland, 1996).

Disease prevention and treatment via good husbandry and biosecurity management practices that reduced stress should be a priority and management should be based on research focused on reducing disease occurrence and increasing disease resistance. Many studies on good production management and welfare have already been conducted in the aquaculture industry; however, this has occurred to a lesser extent within the ornamental fish industry. In the pet fish industry, multiple stressors are found in all parts of
the supply chain that contribute to poor welfare, increased disease prevalence, and higher mortality. Pet fish stores are the final segment of the trade chain trade and play an important role in ensuring good quality fish production. Intervention practices that improve welfare that have been adopted by the aquaculture industry for several decades have only partially been adopted within the ornamental fish industry.

From 1985 to the present day, there has been a lack of scientific literature directly revealing the state of ornamental fish welfare. Although knowledge of management practices and interventions used to increase production and improve fish quality of life and welfare has been available for several decades, these have only partially been considered and adopted within the ornamental fish industry. Moreover, some practices used in aquaculture fish are uncommon but are very important, such as using an appropriate handling technique (Sneddon, 2009; Rose et al., 2014; Stevens et al., 2017). One big factor when considering appropriate ornamental fish management is determining the number and diversity of species traded. This is the reason why using research related to reduced stress and improved welfare for one species of fish is questionable when applied to another fish species (Huntingford et al., 2006; Stevens et al., 2017). Thus, there is a need to increase the amount of information collected from people who have experience on the entire ornamental fish supply chain, from upstream to downstream levels, to design specific requirements and guidelines for fish management and create a welfare assessment for different pet fish species.

Furthermore, there is a great need to collect more accurate statistics, develop improved husbandry standards, and conduct more extensive research to improve the management and welfare standards within the industry. This will increase the marketability of the hobby, potentially translating into greater profits as well as improved living conditions for the animals.

**CONCLUSION**

Fish welfare is defined in term of quality of life that mean fish has “good health” and can obtain “what it wants” and also included natural behavior and is not experiencing negative emotions. This approach has already been used to improve the aquaculture industry, however this approach has been adopted to a lesser extent within the ornamental fish industry even through the supply chain involves multiple stressors that can result in poor welfare, increased disease prevalence and death. Our study is the first to investigate management of ornamental fish stores in Chiang Mai province and examine perceptions and knowledge of owners about fish diseases and drug usage that affects ornamental fish health and welfare. The results from our studied indicate extremely poor animal welfare and poor husbandry in term of fish health. Thus, the information gained from this study can be used to develop further studies that concentrate identifying stressors and practices that affect pet fish welfare in stores as well as in the other sections of their supply chain.
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AUTHOR CONTRIBUTIONS

Project administration and conceptualization, B.S. and S.P.; methodology, investigation and validation, B.S., V.P. and S.P.; data curation, formal analysis and visualization, B.S., R.M., W.C. and S.P.; original draft preparation, B.S. and S.P.; review, editing and approval of final draft, J.K.B. and S.P. All authors contributed to the critical review and revision of the manuscript and have approved the final version.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Assefa, A., Abunna, F., 2018. Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. Vet. Med. Int. 10, 5432497.

Attia, J., Millot, S., Di-Poi, C., Bégout, M.L., Noble, C., Sanchez-Vazquez, F.J., Terova, G., Saroglia, M., Damsøgård, B., 2012. Demand feeding and welfare in farmed fish. Fish Physiol. Biochem. 38, 107-118.

Bovenkerk, B., Meijboom, F.L.B., 2013. Fish welfare in aquaculture: explicating the chain of interactions between science and ethics. J. Agric. Environ. Ethics. 26, 41–61.

Brown, C., 2015. Fish intelligence, sentience and ethics. Anim. Cogn. 18, 1–17.

Choo, P.S., Williams, M.J., 2003. Fisheries production in Asia: its role in food security and nutrition. Naga. 26, 11-16.

Clements, H., Valentin, S., Jenkins, N., Rankin, J., Baker, J.S., Gee, N., Snellgrove, D., Sloman, K., 2019. The effects of interacting with fish in aquariums on human health and well-being: A systematic review. PLoS One. 14, e0220524.

Culp, S.J., Beland, F.A., 1996. Malachite green: a toxicological review. Int. J. Toxicol. 15, 219-238.

Department of fisheries, Chiang Mai Province. [cited 2018 June 10]; Available from: https://www.fisheries.go.th/fpo-chiangmai/web2/

Falahatkar, B., Poursaeid, S., Shakoorian, M., Barton, B., 2009. Responses to handling and confinement stressors in juvenile great sturgeon Huso huso. J. Fish Bio. 75, 784-796.
General information of Chiang Mai Province, Thailand. [cited 2020 April 20]; Available from: http://www.chiangmai.go.th/managing/public/D8/8D01Feb2017150134.pdf.

Goessling, W., Sadler, K.C., 2015. Zebrafish: an important tool for liver disease research. Gastroenterology. 149, 1361-1377.

Good aquaculture practices for ornamental freshwater for ornamental freshwater. [cited 2020 June 9]; Available from: https://www.acfs.go.th/standard/download/eng/GAP_FRESHWATER_ANIMALS_FARM.pdf

Gopakumar, G., 2016. Water quality management for production of freshwater ornamental fish. In: Best management practices for freshwater ornamental fish production. New Delhi, India: National Fisheries Development Board, Hyderabad.

Hoque, F., Hussan, A., Das, A., Chakraborty, P., 2018. Managing water quality and fish health in aquaculture: Farmer’s traditional practices in west Bengal. Int. J. Fish Aquat. Stud. 6, 31–35.

Huntingford, F.A., Adams, C., Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandoe, P., Turnbull, J.F., 2006. Current issues in fish welfare. J. Fish Biol. 68, 332-372.

Huntingford, F.A., Kadri, S., 2014. Defining, assessing and promoting the welfare of farmed fish. Rev. Sci. Tech. Off. Int. Epiz. 33, 233-244.

Islam, S.B., Habib, M., 2013. Supply chain management in fishing industry: A case study. Int. J. Supply Chain Mgt. 2, 40–50.

Kosemani, S.E., Adewole, H., Olaleye, V., 2017. Assessment of effect of fish feeding practices on the water quality of some fish pond in Ado Ekiti, Nigeria. Int. J. Fish Aquat. Stud. 5, 357-564.

Leung, T.L.F., Bates, A.E., 2013. More rapid and severe disease outbreaks for aquaculture at the tropics: Implications for food security. J Appl. Ecol. 50, 215-222.

Liang, J.Y., Chien, Y.H., 2013. Effects of feeding frequency and photoperiod on water quality and crop production in a tilapia-water spinach raft aquaponics system. Int. Biodeterior. Biodegrad. 85, 693–700.

Magada S., Mercy, T.V.A., 2016 Health Management in Ornamental Fish Farming. In Best management practices for freshwater ornamental fish production. New Delhi, India: National Fisheries Development Board, Hyderabad.

Malindretos, G., Vlachos, I., Manikas, I., Chatzimanolakis, M., 2016. Future prospects of sustainable aquaculture supply chain practices. Smart Innov. Syst. Technol. 52, 487-497.

Mather, J.A., 2019. Ethics and care: For animals, not just mammals. Animals. 9, 1–12.

Midtlyng, P.J., 1997. Vaccinated fish welfare: protection versus side-effects. Dev. Biol. Stand. 90, 371-379.

Miller-Morgan, T.J., Glaze, D., Heidel, J., 2012. Pet fish medicine and health management in the ornamental fish industry. In proceeding of the international conference on sustainable ornamental fisheries- way forward. Cochin, India: SOFI-WF.

Noga, E.J., 2010. Fish Disease: Diagnosis and Treatment. NJ, USA: A John Wiley & Sons, Inc., Publication.

Oladele, O.O., Olarinmoye, A.O., Ntiwunka, U.G., Akintomide, T.O., 2015. Survey of bacterial isolates from cases of fish disease outbreaks and their antibiotic susceptibility patterns. Nig. J. Fish Aqua. 12, 901-906.

Ornamental Fish and Aquatic Plant Thailand Directory 2011-2012. [cited 2020 April 12]; Available from: https://www.fisheries.go.th/aquaoma/web2/images/download/institute%20in%20D.pdf.

Pikulkaew, S., Meeyam, T., Banlunara, W., 2009. The outbreak of koi herpesvirus (KHV) in koi (Cyprinus carpio koi) from Chiang Mai province, Thailand. Thai J. Vet. Med. 39, 53-58.

Pikulkaew, S., Phatwan, K., Banlunara, W., Intanon, M., Bernard, J.K., 2020. First evidence of carp edema virus infection of koi Cyprinus carpio in Chiang Mai province, Thailand. Viruses. 12, 1-11.

Pleeging, C.C.F., Moons, C.P.H., 2017. Potential welfare issues of the Siamese fighting fish (Betta splendens) at the retailer and in the hobbyist aquarium. Vlaams Diergeneesk. Tijdschr. 86, 213-223.

Qi, W., 2002. Social and economic impacts of aquatic animal health problems in aquaculture in China. FAO Fish Tech. Pap. 2, 55–61.
Saengsitthisak, B., Chaisri, W., Punyapornwithaya, V., Mektrirat, R., Klayraung, S., Bernard, J.K., Pikulkaew, S., 2020. Occurrence and antimicrobial susceptibility profiles of multidrug-resistant aeromonads isolated from freshwater ornamental fish in Chiang Mai province. Pathogens. 9, 1–13.

Sahoo, P.K., Swaminathan, T.R., Abraham, T.J., Kumar, R., Pattanayak, S., Mohapatra, A., Sánchez-Suárez, W., Franks, B., Torgerson-White, L., 2020. From land to water: Taking fish welfare seriously. Animals. 10, 1–11.

Sneddon, L.U., 2009. Pain perception in fish: indicators and endpoints. ILAR J. 50, 338-342.

Stevens, C.H., Croft, D.P., Paul, G.C., Tyler, C.R., 2017. Stress and welfare in ornamental fishes: what can be learned from aquaculture? J. Fish Biol. 91, 409-428.

Stevenson, M., with contributions from Nunes, T., Heuer, C., Marshall, J., Sanchez, J., Thornton, R., Reiczigel, J., Sebastiani, P., Solymos, P., Yoshida, K., Jones, G., Pirikahu, S., Firestone, S., Kyle R., Popp, J., Jay, M., Reynard, C., 2019. epiR: Tools for the analysis of epidemiological data. R package version 1.0-10. 2019. [cited 2020 November 1]; Available from: https://CRAN.R-project.org/package=epiR

Sung, Y.Y., MacRae, T.H., Sorgeloos, P., Bossier, P., 2011. Stress response for disease control in aquaculture. Rev. Aquac. 3, 120-127.

Talbot, C., Corneillie, S., Korsoen, O., 1999. Pattern of feed intake in four species of fish under commercial farming conditions: implications for feeding management. Aquac. Res. 30, 509-518.

Tavares-Dias, M., Martins, M.L., 2017. An overall estimation of losses caused by diseases in the Brazilian fish farms. J. Parasit. Dis. 41, 913-918.

Verner-jeffreys, D.W., Welch, T.J., Schwarz, T., Pond, M.J., Woodward, M.J., Haig, S.J., Rimmer, G.S.E., Roberts, J., Sebastiani, P., Solymos, P., Yoshida, K., Jones, G., Pirikahu, S., Firestone, S., Kyle R., Popp, J., Jay, M., Reynard, C., 2009. High prevalence of multidrug-tolerant bacteria and associated antimicrobial resistance genes isolated from ornamental fish and their carriage water. PLoS One. 4(12), e8388.

Videira, M., Velasco, M., Malcher, C.S., Santos, P., Matos, P., Matos, E., 2016. An outbreak of myxozoan parasites in farmed freshwater fish Colossoma macropomum (Cuvier, 1818) (Characidae, Serrasalminae) in the Amazon region, Brazil Aquac. Reports. 3, 31-34.

Watts, J.E.M., Schreier, H.J., Lanska, L., Hale, M.S., 2017. The rising tide of antimicrobial resistance in aquaculture: Sources, sinks and solutions. Mar. Drugs. 15, 1–16.

Yildiz, H.Y., Robaina, L., Pirhonen, J., Mente, E., Domínguez, D., Parisi, G., 2017. Fish welfare in aquaponic systems: Its relation to water quality with an emphasis on feed and faeces-A review. Water. 9, 1–17.

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