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Quantitative measurement of epithelial injury in ornamental silver dollar fish (*Metynnis orinocensis*) captured in the wild, imported wild-caught, and aquacultured

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Abstract. Skin injuries in ornamental fishes can compromise their overall health and quality. A fluorescein test coupled with computer-aided color analysis was used to quantify the extent of skin injuries on the ornamental silver dollar fish (*Metynnis orinocensis*) monitored at several points during transportation from collection in the wild in Colombia, to post import at a wholesale facility in Miami, USA, to fish cultured and handled on a farm. After capture and handling, all the fish had minor skin wounds and the extent of injuries covered 3.5-4.6% of their body surface, regardless of fish size. Fifteen percent (15%) of the silver dollars captured in the wild showed skin injury immediately after collection. The incidence of skin injures was significantly lower in the group from the aquaculture pond (5%) and was observed to be greatest in fish after import (57%). Farmed ornamental fish may be preferred as fewer of them exhibit skin lesions after handling.
Key words: aquaculture, aquarium fish, skin lesions, fluorescein, color analysis.

Resumen. Lesiones de la piel en los peces ornamentales pueden poner en peligro la salud general y la calidad ornamental de estos. Una prueba de fluoresceína junto con análisis de color asistido por ordenador se utilizó para cuantificar la magnitud de lesiones en la piel del pez ornamental moneda (*Metynnis orinocensis*) incurridas durante el transcurso de su comercialización, desde su captura en la naturaleza en Colombia hasta un establecimiento mayorista de importación en Miami, Estados Unidos, y en aquellos peces cultivados en una granja. Después del manejo de captura, todos los peces tuvieron heridas menores en la piel y la extensión de las lesiones cubría 3,5-4,6% de la superficie corporal. Se demostraron lesiones en la piel en el 15% de los peces inmediatamente después de la colecta en la naturaleza. La incidencia de lesiones cutáneas fue menor en el grupo de peces provenientes de la acuicultura (5%) y se observó ser mayor en el grupo de peces en lugar de arribo (57%), despues de la etapa de importación. Los peces ornamentales provenientes de cultivo podrían ser preferidos a los capturados en la naturaleza ya que un número menor de ellos presentan lesiones en la piel.
Palabras clave: acuicultura, peces de acuario, lesiones de la piel, fluoresceína, análisis de color.

Introduction. The ornamental fish industry relies on the fish product reaching the market alive, in good health, and of sufficient visual quality to attract both advanced and novice aquarists, after withstanding the stresses and injuries during collection, storage, shipping and handling. At each step in the trade route there is considerable moving and handling of the fish. Almost any type of net, container, or bowl, and even bare hands, are used to handle the fish. Hand nets, of all different shapes and sizes are one of the most widely used tools for collecting and moving ornamental fish. However, few studies have documented the injuries associated with the collection, handling, and shipping of ornamental fishes. Many of the existing studies have focused on the possible morbidity and mortality that can be incurred during handling and transport (Chao et al 2001; Gil & Martinez 2001; Vagelli &

Erdmann 2002; Cato & Brown 2003; Lunn & Moreau 2004; Lim et al 2003). The journey of collection and storage, through transport and distribution, to their final location in the hobbyist tank, may cause severe stress to the fish and may also result in their mortality (Lim et al 2007). The necessity to maintain and transport fishes alive has prompted concern over quality control and increased focus on non-destructive fishing practices (Sadovy & Vincent 2002).

Studies on non-ornamental fish show that net handling can cause many physical and physiological problems as well as endocrine imbalances (FSBI 2002; Conte 2004; Brydges et al 2009). Roughly netting the fish or using an abrasive net will easily remove fish scales and disrupt the protective mucus coat (FSBI 2002; Conte 2004). Also, if many fish are handled at the same time they may injure themselves inadvertently as there is the potential to cause a deep cut or puncture to the skin with their fin rays or spines. Fins and eyes can also suffer damage as a result of rough handling (Barthel et al 2003). Acute cutaneous injuries caused by abrasions and rough handling have been known to significantly increase the ability of pathogens to cause disease in the afflicted fish (Bader et al 2006).

The traditional method used to discern skin injury in fish is by clinical examination aided by a standard body injury assessment chart with a diagram of the external anatomy of the fish. Anatomical diagnostic charts have been designed to function as a general reference to identify where a readily apparent external skin lesion may be located on the fish and if there is some obvious body part missing (Goede & Barton 1990; Kane et al 1999; Kane 2005). Such clinical assessments can also be expressed in quantitative terms using a health assessment index (HAI) where measured health variables such as damage to skin, body condition factor, and blood parameters like hematocrit are assigned index values or numerical ratings from best to worst or the like (Adams et al 1993). However, it still remains difficult to conduct an accurate health or clinical assessment, especially for those injuries that are not yet visible or apparent in the skin that can serve as a portal or point of infection for more severe forms of a disease. The error in diagnostic accuracy may also be compounded when handling fish of small size.

The purpose of this study was to determine the prescence and extent of skin injuries incurred during normal handling procedures of the silver dollar fish (*Metynnis orinocensis* (Steindachner, 1908) at different stages in the supply chain from capture, to post import at a wholesale facility, to fish cultured on a farm. Silver dollars are amongst the most popular and valuable of ornamental fishes from South America, and are in high demand the world over. Colombia has been a world leader in ornamental fish exports and the largest exporter of wild-caught ornamental fishes to the USA (Chapman et al 1997; Chapman 2000). Silver dollars or, "monedas" as they are regionally called, are among the principal species exported from that country (Gil & Martinez 2001). Therefore, Colombia and silver dollars were chosen as they represented a typical fish in the market, employing standard practices for their collection from the wild and subsquent handling.

We used the fluorescein test described by Noga & Udomkusonsri (2002) to detect any breaks in the skin, and computer-assisted image analysis of digital photographs to determine the spatial extent of the injury (Wallat et al 2002; Davis & Ottmar 2006). The fluorescein test can be performed on live fish, and can discern low level injuries of the skin (Noga & Udomkusonsri 2002). Understanding how and when injuries to the skin occur are important for maintaining the health and overall quality of ornamental fish in the trade. Skin injuries will cause a significant reduction in the commercial value of ornamental fishes, which are traded or selected primarily for their overall appearance. Skin injuries may also may compromise the overall health of fish. Even when not visibly apparent or obviously threatening, superficial skin injuries can increase the risk of infection. Importantly, fish handling and skin injury can lead to delayed mortality (Svendsen & Bøgwald 1997; Davis 2005), this being more probable given the relatively small size of most ornamental fish. Delayed mortality rates in ornamental fishes caused by improper collection and handling methods may represent a 'silent' threat that can influence the sustainability of natural populations of fish destined for the aquarium trade. Establishing more accurate estimates of skin injuries may point to possible relationships between them and net handling procedures during collection, holding, and transport of ornamental fishes. Results can provide a useful management tool for the conservation of the resource and its sustainability in the market.

Materials and Methods. Silver dollars or 'monedas', (*M. orinocensis*) were collected in the wild along the shallow water fringes of flooded palm forests known as 'morichales', in a region known as the llanos, near the town of Puerto Gaitan, Colombia. Many other species of ornamental fish are also collected from these flooded forests during the rainy season. These areas are very remote and require travel for most of the day by truck, then hiking to the collection site. The fish were collected with a seine net made of nylon fiber with a mesh size of 3 mm; a typical method of capture by fishermen. After capture, fish were placed in assorted sizes and types of plastic containers filled with surrounding ambient water. Within an hour of collection, fish were sorted into polyethylene bags filled with water and oxygen (approximately 25 fish per bag), and transported via truck to a holding facility in the city of Villavicencio. However, motorcycles are the most common vehicles used for transporting the fish from collection sites to the holding and culture facilities. The entire process including collection and transport to the holding facility took approximately 48 hours. After the initial capture, all sampled fish were manipulated with fine mesh hand-nets typically used in brine shrimp culture.

The fish holding facility in Villavicencio served as a fish collection center where artisanal fishermen took their fish for later distribution to wholesalers and retailers. Silver dollars were also farmed for export at the center. They were raised in earthen ponds (5.2 x 4.2 m) at a density of some 10,000 fish per pond, and were fed sporadically with commercial pellets used for feeding tilapia. The farmed fish were sampled from the pond with the same seine net used for the collection of fish from the wild. A different stock of silver dollars, also collected from the wild and exported from Colombia, was sampled at an import wholesale facility in Miami, USA.

To examine the fish for skin injuries we updated a computer-assisted color image analysis system that we utilize in our laboratory to objectively measure the skin color in live ornamental fish (Wallat et al 2002). For this study the fish were photographed under white light (6500K) and UV (254 nm) light conditions. The extent of wounding to the dermis was quantified using fluorescein, which is a compound that perfuses across any break in the dermis and can be visualized using the UV light (Noga & Udomkusonri 2002).

A portable photography studio (BestLight Studio) illuminated from above with a circular fluorescent light tube was used to control light exposure, and create an environment suitable for taking fluorescent photographic images. When photographed in the dark, the studio light was turned off, and the inside and outside of the studio was covered in black felt. The fish were then illuminated with a handheld UV lamp (model UVGL-58; UVP), held at a 45^o angle so the fluorescein dye will emit fluorescence brightly wherever it was present. A consitant angle was critical to taking good images with maximum flourescence detection. Photographs were taken with a Nikon 4500 digital camera with Bower +4 macro (close-up) 28 mm lens. To take the UV photographs, no light filter was used, and the camera was set to manual exposure mode, ISO of 200, lens aperture value of F5.7, and shutter speed of 1 sec. Camera distance to the fish had little effect on fluorescence detection only on focus, however, the fish were photographed from a consistent distance.

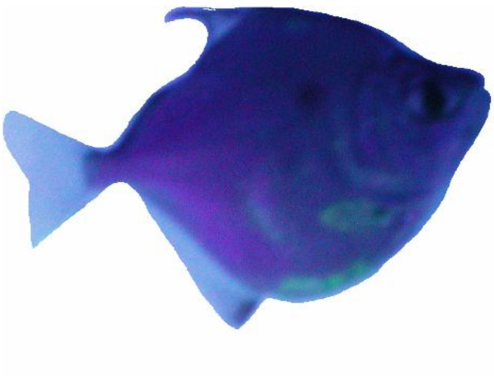
In preparation for the test, a stock solution (200 g mL^{-1}) of water soluble fluorescein sodium salt (F6377; Sigma-Aldrich) was prepared in a plastic 1.4 L tank. Batches of 5 fish were immersed in the fluorescein solution for 6 min, and rinsed with freshwater three times for 2 min apiece in a similar but separate tank; the water was exchanged after each rinsing. To accommodate and pose the fish for a photograph, they were placed sideways on a foam-backed bottom of a 100 mm diameter Petri dish. The cover of the dish served as restrainer and to calm the fish. No anesthesia was used; MS-222 is the only anesthetic agent FDA approved for aquaculture but is known to interfere with the fluorescein test (Davis & Ottmar 2006). When the fish stopped moving temporarily, the lid was removed and the picture taken quickly; the procedure taking no more than 1 min. Afterwards the fish were placed in a tank filled with well-aerated water (DO 5-7 g mL^{-1}) for recuperation and observed for 2 days. The fish were photographed out of the water since the water attenuated and scattered the UV light, and interfered with detection of the fluorescence.

Digital images were downloaded from the camera to an IBM Thinkpad Lenovo X61 computer and stored in bmp file format. The Microsoft Paint software (Microsoft Corp. Windows XP version) was used to trace the outline of the image of the fish, and the color analysis LensEye software (Version 10.1.7) to permit edge detection and quantify the amount of color in the digital color images. Not only did the presence of fluorescence in the digital color images indicated the occurrence of skin injuries but allowed for edge detection. Edge detection refers to a clear and objective demarkation of abrupt changes in pixel intensity, which characterized the boundaries of the skin lesions in the color images and permitted estimation of the overall extent of the injuries. Both sides of the fish were photographed to estimate approximately the percentage of skin injury covering the body.

The quantitative variables of weight, length, size, and percentages of skin injury were presented as mean ± standard deviation. Quantitative estimates of the presence and extent of skin injury in the groups sampled were analyzed using a Chi-square test at a 99% level of confidence. The quantitative analyses were performed in a personal computer using the statistical software JMP 9 (SAS Institute, Version 9.0.0).

Results and Discussion. The silver dollar fish collected directly from the wild and sampled on site (n= 20) were 1.6 ± 0.5 g BW and 42.6 ± 4.6 mm TL. The aquaculture fish sampled directly on farm (n= 20) were 0.5 ± 0.4 g BW and 28.7 ± 6.2 mm TL. The wild-caught silver dollars sampled at the import wholesale facility (n= 20) were 5.9 ± 2.4 g BW and 52.8 ± 6.6 mm TL. When fishing for the silver dollars in the wild, net seining caused significant mortalities. In contrast, no immediate mortalities were observed in the silver dollars harvested from the aquaculture pond, nor those imported. All live fish, after capture and handling, had minor skin wounds and the overall extent of injuries covered 3.5-4.6% of their body surface; across study groups and regardless of fish size. Fifteen percent (15%) of the silver dollars captured in the wild showed skin injuries immediately after collection. At the wholesale facility, 57% of the imported wild-caught silver dollars had skin injuries. The lowest number of injured fish (5%) was found in the group sampled at the aquaculture farm which were also the smallest in overall size. This finding is counter intuitive to the idea that organisms of a smaller size would have larger surface area contact with the net or handling and therefore greater potential for injury or trauma. These findings suggest that the source or where the fish were coming from influenced the presence or absence of injury.

Although it was expected that some injury would always occur whenever fish are captured and handled, a consistent pattern of abrasion was observed at the import wholesale facility for the fish that were recently received from shipment. Most prominent were traces of fluorescence seen along the ventral surface of the body indicative of mild skin abrasion, probably caused by the net (Figure 1a). Silver dollars posses a laterally compressed body (flattened side to side), with a pronounced ventral keel. The ventral keel is a hardened ridge extending from the ventral surface of the fish, from just below the gill plate region towards the anal fin. This places their body weight entirely along the keel and positions their body to be held inadvertently along their laterally-flat sides when manipulating them with a hand net; the net causing the wound by scraping the mucous or skin. When observed grossly the ventral keel area had missing scales and appeared to have deeper tissue injuries (Figure 1b). The high number of fish packed for shipment, approximately 200 fish per bag, can exacerbate this type of injury. To alleviate the possible consequences of this practice, densities should be reduced thereby reducing the number of fish to rub against each other. A much more viable alternative and one that can substantially increase the overall health of the fish is to hold or acclimatized the fish for a few days after capture to allow for rapid healing of superficial injuries. During this time theraputics can be used to prevent infection of areas exposed to trauma. While in recuperation, they can be fed lightly with addition of a nutritional supplement such as vitamin C, which has been shown to significantly reduce mortality post-shipment and improve overall health (Lim et al 2002).



a.

b.

Figure 1. Wound pattern as a result of shipping and representative color analysis. Image (a) represents the fluorescein stained individual with the pattern of injury noted in the fish that were shipped from Colombia to USA wholesaler. There is a line of fluorescence seen on the ventral keel, which can also be seen grossly in control image, where the fish appears to be missing scales and having a deep injury in the dermis (b).

Of the 20 fish sampled from the fish group acquired from the aquaculture farm, only one individual showed significant markings that were detected by the fluorescein test. Fish from this group lived confined at higher densities and were captured with same mesh net as those collected in the wild. The lack of injury prescence suggests that fish were subject to less handling and had more ability to withstand injury or trauma. It appears that aquaculture fish may be less prone to skin lesions than wild fish. The lack of injury presence further support the suggestion that the culture of ornamental fish provides a viable alternative to the collection of fish from the wild and serves as a useful management tool to maintain the sustainability of the resource, especially whenever fish are being caught indiscriminately, which is often the case when fish are in popular demand and found primarily in the wild.

The fluorescein test has been used to understand the process of disease and healing in fish, the possible sources of skin damage in fish associated with different handling methods, and skin sensitivity after exposure of the skin to assorted chemicals (Noga & Udomkusonsri 2002; Davis & Parker 2004; Fontenot & Neiffer 2004; Davis et al 2008; Colotelo & Cooke 2011; Ibrahem & Mesalhy 2010). Most often used by the ophthalmic community as a clinical diagnostic tool for viewing the integrity of the cornea, there is very little research on the exact function of the interaction of ocular cells with the dye (Morgan & Maldonado-Candina 2009). As a relatively new tool for fish diagnostics, further research is necessary to determine how fluorescein adheres to damaged sections of the fish skin and consistently yields fluorescence. Fluorescein detects obvious skin abrasions, ulcers, and fin fraying in fishes (Noga & Udomkusonsri 2002; Davis & Ottmar 2006; Colotelo 2009). In this study it was observed that fluorescein did not stain all visible injuries like those areas where some of the scales were missing from the body surface. Further experiments using *M. orinocensis* made with a superficial injury to the dermis from direct scale removal, revealed that this type of injury has a detection period of less than 48 hours, as the injured areas were reduced in size by half within 24 hours (unpublished data). This could be attributed to a rapid process of reepithelialization that can begin within an hour of injury. During the healing process there is rapid proliferation, organization, and differentiation of cells; the restoration of all cell layers typically completed at 4 to 6 d postinjury (Fontenot & Neiffer 2004). Therefore, the fluorescein test is a useful tool for detection of superficial acute injury to the dermis and other deeper tissue traumas.

Only 3 out of 60 fish died after the fluorescein test. However, these individuals displayed large areas of fluorescence (14% or more of their body surface) and gross morphology of abrasions, attributed to their prior handling during capture or shipment. The fluorescein test, especially in combination with computer-aided color analysis, is a useful tool for rapid assessment of acute injuries in fish that do not require killing of the fish. This test is particularly suited for the ornamental fish industry, especially in time periods of concern: collection, shipping, sorting and the like.

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