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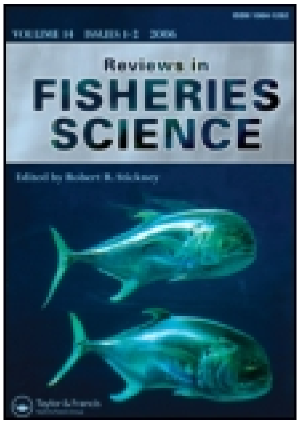

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Traceability Issues in the Trade of Marine Ornamental Species

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Traceability Issues in the Trade of Marine Ornamental Species

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In the last decade, the trade of marine ornamental species has experienced a significant expansion worldwide; however, this industry still relies on a large number of unsustainable practices (e.g., cyanide fishing, overexploitation of target species) and needs to shift its operations urgently to avoid collapsing. Under this scenario, traceability and certification emerge as important management tools that may help this industry to shift toward sustainability. This industry relies on the trade of thousands of small-sized species that are traded live on a unitary basis with high market value. These features, along with a fragmented and complex supply chain, make the traceability of marine ornamental species a challenging task. This study presents the most commonly used methods to trace aquatic organisms and discusses their suitability to trace marine ornamental species. The use of bacterial fingerprints appears to be the most promising method to successfully trace marine ornamentals, but it is most likely that a combination of two or more traceability methods need to be implemented to cover all the unique features displayed by the live trade of marine ornamental species.

Keywords traceability methods, marking methods, certification, sustainability, supply chain

1. OVERVIEW OF MARINE ORNAMENTAL SPECIES TRADE

Marine organisms have long been traded as ornamentals worldwide and currently supply three distinct markets: the curio/home décor, the jewelry industry, and the aquarium trade (reviewed by Thornhill, 2012). The first two markets rely on the trade of dead animals (e.g., crude or carved coral skeletons, mollusc shells, and dried fish; Bruckner, 2005; Tsounis et al., 2010), whereas the marine aquarium industry trades a multitude of live invertebrates and fish, mostly captured from coral reefs (Wabnitz et al., 2003). In the present work, the term "marine ornamentals" will be used sensu stricto only referring to organisms employed to supply the marine aquarium trade.

When one thinks about marine ornamentals, the first image that comes to mind is the colorful coral reef wildlife. Color, however, is not the only feature that makes a marine organism suitable as an ornamental species. Marine organisms that provide a service in home reef aquariums (e.g. algae grazers, fish-cleaners, and species controlling the growth of "nuisance organisms") are also heavily collected from the wild and sold in the marine aquarium trade (Rhyne et al., 2009). Marine aquarium keepers also look for animals that display mimetic adaptations, associative behavior, and that are able to thrive in captivity without harming other tank inhabitants (a feature commonly termed as "being reef safe"; Calado, 2006).

The trade of marine ornamentals began in the 1930s, with Sri Lanka being one of the first countries to collect and export live reef fish (Wood, 2001). During the 1950s, the global trade of marine ornamentals started to increase globally with the shipping of live fish by air (Wood, 2001). In the 1990s, with the advent of new marine aquarium technology, hobbyists started shifting their preferences from fish-only tanks to displays truly mimicking coral reef ecosystems (e.g., displaying fish and live invertebrates, namely corals; Wabnitz et al., 2003; Rhyne et al., 2009). This shift in the marine aquarium trade promoted a sharp increase in the popularity of invertebrate marine ornamentals.

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By the early 2000s, this business was already a multi-million dollar industry that mostly harvested wild specimens from coral reefs in the Pacific (mainly from the Philippines and Indonesia) and exported them worldwide, mainly to the United States, E.U. countries, and Japan (Green, 2003; Olivier, 2003).

The accurate quantification of the volume and value of the trade of marine ornamentals is a challenging task due to the large number of species traded (Wabnitz et al., 2003; Tissot et al., 2010) and to the significant amount of illegal, unreported, and unregulated fishing practices (Thornhill, 2012). From May 2004 to May 2005, the United States alone imported over 1,800 fish species (Rhyne et al., 2012b). In the same study, the authors reported that values on shipment declarations exceeded 11 million marine ornamental fish but matched those of attached commercial invoices in only 52% of the cases. It is unquestionable that millions of marine ornamental fishes are traded every year to supply the marine aquarium industry, but Rhyne et al. (2012b) suggested that prior studies (e.g., Wabnitz et al., 2003; Smith et al., 2008) may have overestimated the true volume of this trade. Nevertheless, the number of fish that are collected from the reef and die along the supply chain prior to export and after import is largely ignored and may be significant (Rubec et al., 2001). The number of traded marine ornamental invertebrates, from corals to several other groups of marine invertebrates (e.g., decapod crustaceans, snails, anemones, and polychaetes) is also impressive, ascending to several hundreds of species and hundreds of thousands of organisms per year (Wabnitz et al., 2003; Jones, 2008; Rhyne et al., 2009; Murray et al., 2012). As for marine ornamental fishes, current data on the number of marine ornamental invertebrates collected from the wild may also be underestimated due to the omission of potential losses through the supply chain.

From ocean to aquarium, marine ornamentals commonly pass through a long, fragmented, and rather complex supply chain. This supply chain is commonly represented by collectors and aquaculturists, middlemen, wholesale exporters and importers, retailers, and hobbyists (Figure 1; Green, 2003; Mathews Amos and Claussen, 2009). Throughout the supply chain, the value of collected animals is invariably inflated, with collectors clearly being the most underpaid players in the trade (Wood, 2001; Wabnitz et al., 2003). Most marine ornamentals are shipped by air in individual plastic bags filled with seawater and oxygen (usually at a proportion of 1:3 of the bag volume), making the freight the most expensive step of trade. Mortality of marine ornamental species along the supply chain has been suggested to vary from a few percent to up 80%, with longer supply chains commonly having higher mortalities (Sadovy, 2002). These numbers could be significantly reduced by shortening the supply chain and using codes of best practices for the collection, shipping, and acclimatization of marine ornamental species, namely the effective banning of destructive fishing practices (e.g., cyanide fishing; Thornhill, 2012). Additionally, such approaches would contribute to decreasing the number of extra specimens that are commonly collected to compensate for the loss of specimens that die along the supply chain.

The true dimension of the ecological impact promoted by this industry is not yet determined. It is accepted that the illegal, unreported, and unregulated capture of marine ornamental species negatively affects marine habitats at various scales (e.g., Barber and Pratt, 1997; Tissot and Hallacher, 2003; Jones et al., 2008; Rhyne et al., 2009). Currently, 95% of all traded specimens in the marine aquarium industry are collected from the wild (reviewed in Thornhill, 2012). Therefore, the aquaculture of these highly priced organisms has been considered as a potential solution to minimize current fishing efforts (Tlusty, 2002; Pomeroy et al., 2006).

The aquaculture of marine ornamental fish and invertebrates has experienced remarkable progress in the latest years, from in situ to ex situ culture; however, several technical bottlenecks are still impairing the commercial scale production of most species (reviewed by Moorhead and Zeng, 2010; Olivotto et al., 2011). Another important constraint for the success of cultured specimens is their higher cost compared to wild-caught animals (Koldewey and Martin-Smith, 2010). Aquaculture, however,

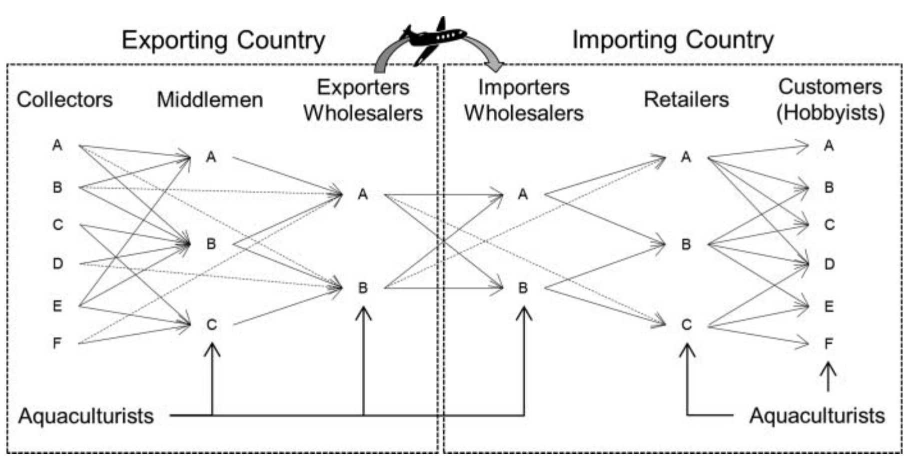

Figure 1 Flowchart of the supply chain in the trade of marine ornamental species (modified from Mathews Amos and Claussen, 2009). This figure is not intended to quantify all the players involved in the supply chain of marine ornamentals.

usually produces specimens more resistant to aquarium conditions and allows the production of species whose collection can be restricted (Wood, 2001). The culture of specimens displaying distinct colors or color patterns from those of wild conspecifics (e.g., "snowflake" clownfish—predominantly white with a few orange blotches) is also gaining popularity in the market, with some varieties reaching 10 to 20 times the value of wild specimens (Olivotto et al., 2011).

2. THE NEED FOR TRACEABILITY IN THE TRADE OF MARINE ORNAMENTAL SPECIES

The negative image currently associated with the collection of live organisms to supply the marine aquarium hobby (Burke et al., 2011) has placed this industry under unprecedented pressure by the media to seek sustainability. It will be impossible to determine the true dimension of this industry and, consequently, its ecological impacts without a reliable way to trace collected specimens along the supply chain. The benefits and costs associated with this global activity may easily be misinterpreted, either from those involved in the trade or those advocating the ban of marine ornamental fisheries.

Traceability protocols are urgently required to pursue ecological, financial, and social sustainability in the trade of marine ornamental species. The popularization of eco-certification of seafood has provided consumers with the chance of making more informed choices on the products they purchase (e.g., seafood originating from sustainable fisheries or aquaculture) (Ward and Phillips, 2008). Eco-certification has also been proposed to help manage the marine aquarium trade (Shuman et al., 2004). At present, marine aquarium hobbyists are still not actively demanding eco-certified ornamental species and, thus, strongly restricting the success of any eco-certification initiative. Another important aspect for the success of eco-certification is the reliability that the eco-label has among the final customer (Wessells et al., 1999, 2001). The ability to differentiate between specimens collected under regulated conditions and those harvested using illegal or destructive fishing methods is still deficient (Wabnitz et al., 2003; Mathews Amos and Claussen, 2009), and therefore, there is a generalized lack of confidence in the credibility of certification. In addition, the collection of marine ornamentals under regulated conditions may not always be sustainable, as certain species can be overharvested easily, even when employing non-destructive fishing methods (e.g., the Banggai cardinalfish, *Pterapogon kauderni* [Kolm and Berglund, 2003] and the yellow tang *Zebrasoma flavescens* [Williams et al., 2009]).

A reliable system for the traceability of marine ornamentals must allow the differentiation between wild specimens and cultured conspecifics (Olivotto et al., 2011). It is also important to determine if traded cultured specimens were bred in captivity or harvested from the wild as larval forms and later grown under aquaculture conditions (Lecchini et al., 2006; Bell et al., 2009). Hobbyists commonly consider cultured marine ornamentals as a sustainable alternative to conspecifics collected from the wild (Alencastro et al., 2005), although it is currently recognized that not all marine ornamentals should be targeted by aquaculture (Tlusty, 2002). The lack of any reliable certification creates an opportunity for fraud, with less scrupulous traders taking advantage of final customers' perception that cultured equals sustainable. A good example is the fragmentation of large coral colonies collected from the wild, whose fragments are later mounted on artificial bases commonly employed in the hobby for coral propagation and then abusively traded as cultured specimens.

The length and complexity of the supply chain, along with poor husbandry practices (from harvesting to handling and holding) is known to promote an increase in marine ornamentals mortality. This scenario generates a positive feedback loop that requires an increase in fishing effort to compensate the losses that occur along the supply chain (Mathews Amos and Claussen, 2009). Therefore, a shorter and more integrated supply chain would result in significantly lower mortality, decreasing the harvest of wild specimens and increasing profitability; ultimately, it would allow better income distribution among all parties involved in the trade. To assume, however, that the most sustainable scenario for the marine aquarium trade would be to produce the most traded species in importing countries, the shorter supply chain option, would be flawed. Such a simplistic approach disregards the financial, social, and environmental impacts that would be promoted on exporting nations by shifting the trade from an extractive to a breeding activity (Tlusty, 2002), even if breeding is intended to be developed at a small scale in exporting countries (Pomeroy and Balboa, 2004). The argument on the ecological footprint associated with the import by air of marine ornamentals from around the globe must also be analyzed with caution, as distance alone is far from being a suitable indicator for carbon emissions, as already highlighted for products for human consumption (food miles concept; Coley et al., 2011). Therefore, aquarium hobbyists will only be able to make a conscientious choice if there is a reliable way to check the animal story ("from ocean to aquarium"), regardless of whether the animal was captured in southeast Asia or cultured in the United States or European Union. The lack of confidence by traders, hobbyists, or both in any part of the supply chain is likely to compromise any certification effort (Mathews Amos and Claussen, 2009), which will be reflected in buyers not being willing to pay extra money for a certified product.

The current lack of control on which and how many marine ornamentals are shipped from an exporting to an importing country, and the impressive number of species and specimens traded, are commonly pointed out as a perfect combination for the introduction of species (Semmens et al., 2004; Bolton and Graham, 2006; Calado and Chapman, 2006; Zajicek et al., 2009). The release of the "killer algae" *Caulerpa taxifolia* in the Mediterranean, Australia, and California (Meinesz and Hesse, 1991; Jousson et al., 2000; Schaffelke et al., 2002); the introduction of the Pacific lion fish *Pterois volitans* and *P. mile* in Florida and the Caribbean (Whitfield et al., 2002; Betancur-R

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et al., 2011); and the introduction of the Indo-Pacific coral, *Tubastraea* spp, in Florida, the Gulf of Mexico, and Brazil (Ferreira, 2003; Fenner and Banks, 2004; Paula and Creed, 2004) are just a few examples of marine ornamental species that have become successful invaders. These species have established thriving populations and continue to expand their range, negatively affecting invaded habitats (Meinesz et al., 2001; Silva et al., 2011b; Green et al., 2012). Curiously, all of these species continue to be traded in the marine aquarium industry, including regions where some of them have already become invasive and/or have legislation limiting or banning their import (Walters et al., 2011; Diaz et al., 2012; Rhyne et al., 2012b). In this way, the enforcement of traceability protocols, coupled with certification programs, can improve the control of export and import animals, restricting the trade of prohibited species. Additionally, traceability might be useful for monitoring invasive species after their accidental or intentional release in the wild, as already applied to trace aquaculture escapees (Hastein et al., 2001).

3. CHALLENGES FOR TRACING MARINE ORNAMENTAL SPECIES

One of the biggest challenges that any traceability effort will face in this industry is the remarkable diversity of species currently being traded as marine ornamentals (over 2,000 different species from a multitude of taxonomic groups; e.g.,Wabnitz et al., 2003; Rhyne et al., 2012a,b). Additionally, a large number of traded species cannot easily be identified to the species level (Green and Hendry, 1999; Smith et al., 2008; Steinke et al., 2009; Murray et al., 2012), which strongly conditions any effort to trace these organisms along the supply chain. This traceability bottleneck has already been acknowledged for hard corals being traded live for marine aquariums (Green and Hendry, 1999); these organisms are protected under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), currently being considered as acceptable to identify most of the species traded for marine aquariums only to the genus level (CITES, 2012). If this is the scenario for a group of heavily monitored marine ornamentals, one may only wonder how challenging it is to monitor the trade of less emblematic species also being heavily collected (e.g., snails and hermit crabs). Another constraint for traceability is the growing popularity associated with the collection and trade of larval and young juvenile ornamental fish and invertebrates (Lecchini et al., 2006; Bell et al., 2009), as their uniform morphology/coloration makes their identification a nearly impossible task.

The growing demand for marine ornamentals worldwide and the shifting trends of which species is "in fashion" in the marine aquarium hobby may also condition the success to effectively tracing these organisms through the supply chain. These dynamics in the trade, along with room limitation in exporting and importing facilities, force most wholesalers to buy from different collectors/middlemen and mix specimens from different origins (Mathews Amos and Claussen, 2009). Additionally, the current lack of financial incentives to separate specimens collected using more environmental friendly approaches (e.g., using hand nets) from those harvested using destructive fishing techniques (e.g., cyanide fishing) has pushed traders to mix all specimens in stocking tanks prior to shipping. This scenario was probably the one that most negatively affected the third-party certification program of the Marine Aquarium Council (MAC). The MAC, a non-government and not-for-profit international organization, aimed to increase sustainability in the marine aquarium trade and consequently assist on marine ecosystem conservation (Holthus, 1999). Unfortunately, the MAC was unable to avoid the abusive use of its certification, allowing less scrupulous companies to "green-wash" their unsustainable fishing and shipping practices. Currently, MAC certification holds little to no credibility among aquarium keepers (Mathews Amos and Claussen, 2009), a feature that compromises the success of any certification program (Ward and Phillips, 2008).

In general, the most significant challenges that need to be overcome by any effort aiming to promote the traceability of marine ornamental species are (1) the complexity of the "typical" supply chain of marine ornamentals; (2) the blurry nature of the trade due to its tradition of illegal, unregulated, unreported, and destructive fishing practices; (3) the lack of an efficient certification program; and (4) a significant unwillingness of the market (from wholesalers to retailers and hobbyists) to pay extra money for certified specimens.

4. TRACEABILITY TOOLS FOR MARINE ORGANISMS AND THEIR POTENTIAL USE FOR MARINE ORNAMENTALS

4.1. Certification and Eco-Labeling

The added value that any product may have from environmental certification or eco-labeling comes from the confidence that the final customer has that the target product is indeed being collected or cultured according to the environmental standards being claimed (Mathews Amos and Claussen, 2009; Tlusty, 2012). Certification and eco-labeling may present potential opportunities for management of wild stocks of marine ornamentals (Shuman et al., 2004; Tlusty et al., 2006), although there must be an incentive for buyers to prefer certified products (commonly more expensive) over non-certified products (Roheim, 2008). A good example on how product price plays an important role on any eco-labeling effort is how easily middlemen and exporters tolerate the trade of cyanide-caught fish (less expensive) instead of solely trading net-caught specimens (more expensive; Rubec et al., 2001). The credibility of any type of certification scheme depends mostly on its independence (who is certifying what, and is the certification party directly involved in the commercial activity), as well as the scientific standards employed to satisfy the environmental claims of the certification and robustness of the chain of custody (Ward and Phillips, 2008).

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In first-party certification, the producer of a given product (e.g., collector or aquaculturist) certifies that its own product meets the standards claimed in the certification, whereas in second-party certification, another interested party (e.g., trade association) certifies the product (Ward and Phillips, 2008). According to the same authors, third-party certification is commonly most reliable, as an independent and accredited entity certifies that the collected or produced product meets the environmental standards claimed by those trading the certified product.

As previously noted, the third-party certification provided by the MAC was never perceived by marine aquarium hobbyists as a synonym of an added value worth paying for at the time of buying marine ornamentals from retailers. The manuals released by the MAC on "Ecosystem and Fishery Management"; "Collection, Fishing, and Holding"; and "Handling, Husbandry, and Transport" (MAC, 2001a,b,c), which provided the core standards for good practices for the different players in the industry, were likely too demanding to be used by poor fishermen communities. This aspect per se would strongly condition the success of this initiative. Additionally, much emphasis on cyanide-free marine ornamental fish was put on the MAC certification. If the approach recently published by Vaz et al. (2012), which described a fast, non-invasive, and non-destructive methodology to detect cyanide caught fish, existed at the time when MAC popularity was at its prime, the success of this third-party certification may have been different. The simple fact of traders knowing that cyanide-caught fish could be identified, with the consequent loss of their MAC certification, would probably have limited the abusive use of the "MAC certified" brand.

The MAC also tried to promote the establishment of a reliable chain of custody in the industry, forcing all organizations and individuals in the supply chain to operate and maintain a documentation system for tracking certified traded specimens back to their collection area or supplier (Holthus, 1999). This is a conceptually appealing idea, but establishing a chain of custody in the trade of marine ornamentals requires that all players in the supply chain must be certified. Those involved in the supply chain, however, may omit or even forge information that can easily compromise the certification. As already noted, the supply chain is highly fragmented, and several players commonly mix certified and non-certified products to fulfill the orders from their customers, a practice that disrupts the chain of custody and threatens the credibility of any certification effort (Mathews Amos and Claussen, 2009).

CITES is another international program that assists regulation of the trade of marine ornamentals (Bruckner, 2001). It is an international agreement that aims to ensure that the international trade of wild animal or plant specimens does not threaten their survival (CITES website, http://www.cites.org/; accessed in September 2012).

The Appendix II of CITES contains a list of species that are not necessarily threatened with extinction, but that may become so if their trade remains unregulated. To trade a species listed in Appendix II, an export permit provided by the CITES Management Authority and Scientific Authority of the country is required (CITES Article IV: Regulation of Trade in Specimens of Species Included in Appendix II, http://www.cites.org/, accessed September 2012). Currently, the only marine ornamentals listed under Appendix II of CITES are stony corals (and live rock), black coral, giant clams, and seahorses. CITES does not have a traceability method by itself and has been facing several problems concerning data discrepancy and reliability (Bruckner, 2001; Blundell and Mascia, 2005). Nonetheless, CITES is committed to develop methods that allow the differentiation of wild-caught corals from captive-bred and captive-reared specimens (CITES, 2002). Unfortunately, the lack of morphological and biological differences between cultured and wild corals makes this a challenging task (Olivotto et al., 2011). So far, most efforts have been focused on hard corals, with the suggested methods to trace captive-bred and captive-reared corals being the use of an artificial base, the incorporation of a barcode or numbered tag (embedded in the coral skeleton as it grows), and the incorporation of dye into the coral skeleton (e.g., alizarin red; CITES, 2002). Unfortunately, none of these methods impairs the illegal trade of wild corals being portrayed as cultured specimens (CITES, 2002).

4.2. Internal Markers

4.2.1. Coded Wire Tags (CWTs)

CWTs are small biocompatible implants that are retained by tagged organisms under the skin (Buckley et al., 1994; Beukers et al., 1995), being made of stainless steel and having a unique binary or numeric code (Hastein et al., 2001). This method has shown a high level of retention and promotes little to no tissue damage in small reef fish (Buckley et al., 1994; Beukers et al., 1995). A public aquarium in the United States has recently introduced the CWT method to monitor longevity of small fish held in captivity and, thus, improved aquarium management (Harmon and Celt, 2012). CWTs were also successfully used in a variety of invertebrate taxa, including decapod crustaceans (Fitz and Wiegert, 1991; Uglem and Grimsen, 1995; Isely and Eversole, 1998; Sharp et al., 2000; Kneib and Huggler, 2001; Davis et al., 2004), bivalve mollusc (Layzer and Heinricher, 2004), and sea urchins (Sonnenholzner et al., 2010). The small size and good applicability in a wide range of marine taxa can suggest that CWTs are good options to trace marine ornamentals; however, from a practical point of view, the suitability of CWTs to the marine aquarium trade is restricted: automatic devices can detect CWT location but they have to be removed and read manually (Hastein et al., 2001). In this way, the information on CWTs can only be retrieved post-mortem (Figure 2(I),a; either death occurs naturally or the target organism is euthanized). Another disadvantage is that this method does not impair the tagging of wild animals as being cultured, or the mislabeling of sustainably collected, or cultured specimens from those originating from unsustainable fisheries or aquaculture.

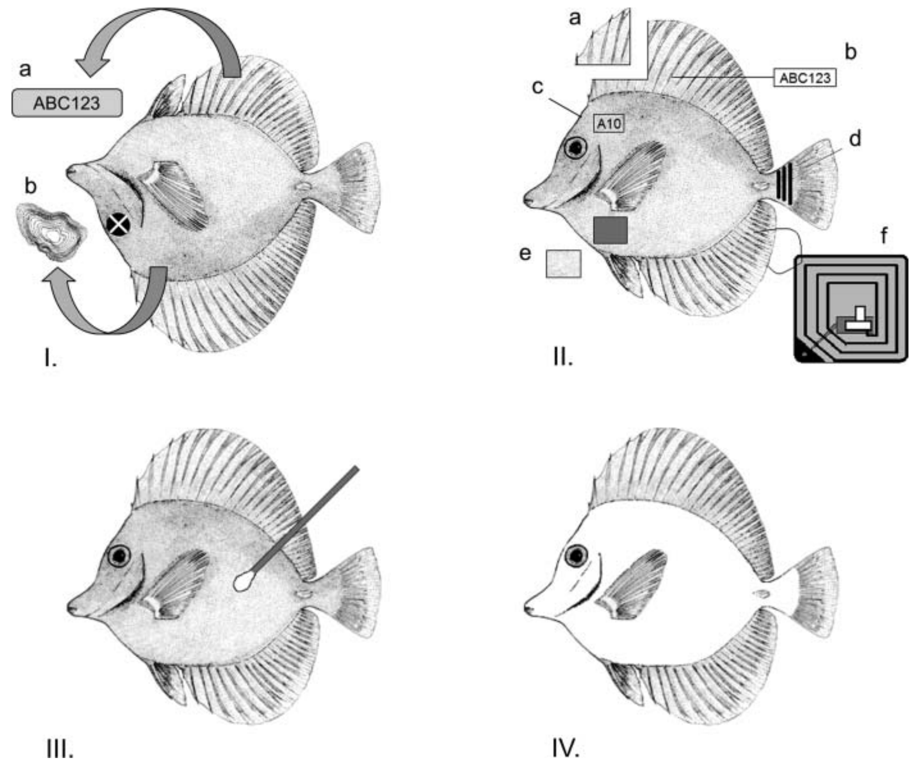

Figure 2 Schematic representation of a common marine ornamental fish (*Zebrasoma flavescens*; modified from Randall, 2001) subjected to different traceability methods. (I) Methods in which data are obtained only after animal's death: (a) coded wire tags and (b) elemental fingerprint (e.g., otoliths). (II) Methods that are either unaesthetic and/or invasive: (a) fin clipping, (b) external tags (e.g., anchor tag), (c) visual implant tag, (d) visual implant fluorescent elastomer, (e) methods that require tissue sample (e.g., fatty acids, stable isotopes, and DNA barcoding), and (f) external tags (e.g., RFID tag). (III) Methods that are not invasive, such as microbiological barcodes (using fish mucus). (IV) Breeding of hybrid specimens (e.g., albino specimens).

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4.2.2. Visible Implants

As the name suggests, visible implants are tags, filaments, or pigments injected under an animal's transparent or translucent skin and are, therefore, externally visible. The two main used methods are the visible implant tags (VITs) and the visible implant fluorescent elastomer (VIE). VITs are small soft tags with an alphanumeric code (Figure 2(II),c), which can have different colors to increase combinations (Hastein et al., 2001). VIEs are soluble polymers that turn into a solid and flexible compound after mixed (Figure 2(II),d; Hastein et al., 2001; Jerry et al., 2001). Different combinations can be made by applying different colors in different body regions. Both VITs and VIEs can be better visualized under fluorescent light. VITs and acrylic paint tag (similar to VIEs) did not affect growth, nor caused mortality, when used in small reef fish (Malone et al., 1999). VIEs have been successfully used with long-term retention and readability in fish (Josephson et al., 2008; Bolland et al., 2009; Soula et al., 2012) and in a variety of juvenile crustaceans, including shrimp (Godin et al., 1996), prawns (Brown et al., 2003; Hung et al., 2012), lobsters (Uglem et al., 1996), crayfish (Jerry et al., 2001), and crabs (Davis et al., 2004). Nevertheless, the retention and readability of visible implants can be compromised in some field conditions, tag location, and growth stage (Jerry et al., 2001; Doupé et al., 2003; Josephson et al., 2008; Bolland et al., 2009). The use of VITs is more recommended when individual identification is necessary due to the higher number of possible combinations that can be achieved (Jerry et al., 2001). Both methods have limited applicability to trace marine ornamental species, as their external visibility might be considered unaesthetic for aquarium keepers. Additionally, these methods would not impair fraudulent traders to tag wild-caught animals as cultured specimens.

4.2.3. Passive Integrated Transponder (PIT)

The PIT has been used to study movements, migration, and behavior of fish (Brannas et al., 1994; Castro-Santos et al., 1996), crayfish (Bubb et al., 2006), sea turtles (Piedra et al., 2007), and other organisms. The PIT tag consists of a microchip, a capacitor, and an antenna coil encapsulated in a small glass cylinder (Roussel et al., 2000). It has no battery, and therefore, energy is provided by a radio-frequency electromagnetic field produced by the reading unit, which transmits the signal with a unique code to the reader (Roussel et al., 2000). This wireless and without contact transmission system is called radio-frequency identification (RFID). On fish, the PIT is commonly injected in the peritoneal cavity with a needle, but for small fish, surgery with suture has shown better retention rates (Baras et al., 1999, 2000). PIT-tagging has shown high and long-term retention rates and has not affected growth or survival on most studied

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species of fish (Baras et al., 2000; Bolland et al., 2009; Younk et al., 2010; Zaroban and Anglea, 2010; Soula et al., 2012), crayfish (Bubb et al., 2002), and anomuran decapod crustaceans (Drew et al., 2012). Acolas et al. (2007), however, suggested that the effect of PIT-tagging on fish mortality may be dependent on species and size. McCormick and Smith (2004) showed that PIT-tagging does not interfere on mortality and growth of small damselfish (40–65-mm standard length), a feature also recorded for other small-sized fish species, such as the Eurasian perch (55–96-mm fork length; Baras et al., 2000), the mottled sculpin (56–85-mm total length; Ruetz et al., 2006), and shorthead sculpin (60–106-mm total length; Zaroban and Anglea, 2010). Tatara (2009), however, reported decreased growth rates for small tagged steelhead (<74 mm), and Soula et al. (2012) reported increased mortality for small tagged red porgy (<10 g). Because most traded marine ornamental specimens are small sized, the physical size of the tag can be an important constraint to the use of the PIT-tagging method. Smaller PITs have already been developed with the advent of new technology (e.g., 8.4-mm-long transponder by Biomark®, http://www.biomark.com, accessed October 2012), although most studies continue to employ 12-mm-long PIT tags on small animals due to the reduced reading distance displayed by smaller tags. Survival rates of tagged small marine ornamental specimens should be investigated further. As visible implants and CWTs, the PIT may still not be reliable if used by less scrupulous traders to tag unsustainable wild-caught animals as sustainable caught or cultured.

4.3. External Markers

4.3.1. Fin Clipping

Fin clipping is a simple and inexpensive method that has been used to mark fish for decades. The marking method consists of clipping fins totally or partially (Figure 2(II),a). It has been commonly used in combination with others tagging methods, namely CWTs and PITs (Ombredane et al., 1998; Bumgarner et al., 2009; Jennings et al., 2009; Hand et al., 2010). Recently, it has been also used as a non-lethal method to collect samples for genetic and biochemical analyses (Valladares and Planas, 2012; Woodall et al., 2012). This method has been mostly used in salmonids, as their adipose fin does not regenerate (Hastein et al., 2001). The use of fin clipping as a marker has many disadvantages, such as limited combinations, identification problems caused by total or partial fin regeneration, and chances of infection with increased mortality (Hastein et al., 2001). Therefore, fin clipping is not recommended to trace marine ornamental species, and fish with injured or absent fins are certainly not desired by marine aquarium keepers.

4.3.2. External Tags

There is a great variety of external tags available, which are basically a bar or a plate with an unique code, attached to the fish body by a nylon or stainless steel wire (Figure 2(II),b; Hastein et al., 2001) or glued on bivalve shells (Hallprint Fish Tagging Solutions®, http://www.hallprint.com, accessed October 2012). RFID tags may also be regarded as external tags, as they have the same operating system of a PIT but are larger and externally attached (Figure 2(II),f). These tags (RFID) have been successfully used to trace live fish traded for human consumption (e.g., groupers; Hsu et al., 2008). External tags are relatively inexpensive and simple to use, although their use for marine ornamental species has clear limitations; the most obvious being highly prized animals losing their attractiveness to buyers once tagged. Additionally, external tags may delay or prevent healing of tagged location as well as increase the chances of infection (Hastein et al., 2001). Hsu et al. (2008) successfully used a wire from the gill to the mouth to tag fish in a non-invasive way, but this option is clearly unsuitable for marine ornamentals.

4.3.3. Thermal and Chemical Branding

The method of thermal and chemical branding consists of physically marking the fish skin, either using heated or cooled tools as well as chemical substances (reviewed in Hastein et al., 2001). Hot branding has used heated metals, soldering irons, NiChrome® electronic devices, and lasers; freeze branding has used lead typewriter letters cooled in a mixture of acetone or ethanol with dry ice and tools using liquid nitrogen (Hastein et al., 2001). Chemical branding can be achieved by "burning" solutions (e.g., silver nitrate and potassium permanganate) or by injection of pigments under fish skin (e.g., alcian blue, hydrated chromium oxide, alizarin complexone, and alizarin red; Hastein et al., 2001). These methods are clearly not suitable for marine ornamentals. Applying thermal or chemical branding in small animals is not only a challenging task and a risk to animal's health, it is also a problem for further identification, as marks can be distorted by fish growth. Additionally, these marking methods would be unaesthetic for marine ornamental species, decreasing their demand and trade value.

4.4. Analytical Methods

4.4.1. Fatty Acids

The profile of fatty acids has been successfully employed to distinguish wild fish from cultured conspecifics (reviewed in Moretti et al., 2003). It has been also used in combination with stable isotopes analyses to determine fish source more accurately (Bell et al., 2007; Busetto et al., 2008). Most cultured fish have significantly higher lipid contents and different fatty acid profile than do wild conspecifics (Moretti et al., 2003). Part of these differences are expected due to the partial replacement of fish oil by plant-derived oils in commercial diets (Moretti et al., 2003) and the fact of fish fatty acid profiles being highly dependent of their dietary lipids (Rosenlund et al., 2001). Therefore, this traceability method would be effective to discriminate between cultured and wild specimens, but it would be ineffective to differentiate marine ornamental aquaculturists that used commercial diets with similar fatty acid profiles (Turchini et al., 2009). Unfortunately, this approach requires the collection of tissue samples (muscle or skin) from the target animal, which is a clear limitation for its use on marine ornamental species (Figure 2(II),e).

4.4.2. Elemental Fingerprint

The chemical composition of calcified structures, such as fish otoliths, mollusc shells, and coral skeletons, can provide environmental signatures that may allow researchers to trace the origin of target animals (Campana and Thorrold, 2001). The elemental composition in these calcified structures reflects the water chemical composition and temperature, therefore enabling the correlation between the animal and its environment (Campana, 1999). Fish otoliths are the most studied structure in elemental fingerprint, mainly because of their continuous growth, age recording feature, and non-susceptibility to reabsorption (Campana, 1999; Campana and Thorrold, 2001; Thorrold et al., 2001). The chemical fingerprint of these structures has already been successfully applied to differentiate fish origin (Tanner et al., 2012; Veinott et al., 2012). Elemental fingerprints have been also successfully used to track the origin of invertebrate larvae by analyzing the exoskeleton of crustaceans (DiBacco and Levin, 2000), as well as mollusc shells (Becker et al., 2005) and statoliths (Zacherl et al., 2003). Elemental fingerprints, however, require post-mortem analysis and, therefore, are not recommended for the trade of marine ornamentals (Figure 2(I),b).

4.4.3. Stable Isotopes

Stable isotope analysis has been successfully applied to distinguish wild from cultured fish, as well as to tell the difference between cultured fish from different farms (Moretti et al., 2003; Dempson and Power, 2004; Bell et al., 2007; Rojas et al., 2007; Turchini et al., 2009; Schroeder and de Leaniz, 2011). The most commonly studied stable isotopes are nitrogen (δ^{15}N), carbon (δ^{13}C), and oxygen (δ^{18}O), which are measured mostly using mass spectrometry (reviewed in Dawson and Brooks, 2001). Carbon and nitrogen are two of the most important elements in the animal structure. These elements are propagated from one organism to another through food assimilation and growth (Rojas et al., 2007). Cultured specimens can be distinguished from wild conspecifics because commercial diets and natural food resources have different ratios of stable isotopes (Rojas et al., 2007). Oxygen (δ^{18}O) can also be used to distinguish cultured fish from different farms, as water resources and geographical location of farms influences the ratio of δ^{18}O in the fish tissue (Turchini et al., 2009). The current use of tissue samples (e.g., muscle, fin, skin, and liver), scale, and otoliths to trace fish and fish products restricts the use of stable isotopes to trace live animals, namely marine ornamental species (Figure 2(II),e). The ratio of stable isotopes in ammonia and feces, however, has been successfully used to study digestibility and protein synthesis in fish (Fraser et al., 1998; Oliveira et al., 2008). Therefore, the possibility to use the ratio of stable isotopes in animals' feces as a non-invasive method to trace live animals should be further investigated. Nonetheless, this analysis would be reliable only for a short period of time, which would be directly proportional to the transit time of food in the animal's gastrointestinal tract. In other words, it would not be possible to distinguish a wild animal if it were fed with commercial diet along the supply chain. Thus, the stable isotope ratio in animals' feces would be best used to distinguish cultured specimens from different farms by linking the animal to a specific commercial diet. The exuviae, in crustaceans, may also be a good solution for non-invasive analysis of stable isotopes and should be investigated further.

4.5. Molecular Methods

4.5.1. DNA Barcodes

DNA barcoding is a taxonomic tool that has been successfully used in the food industry to prevent mislabeling (Smith et al., 2008; Filonzi et al., 2010). This analysis consists of comparing a single gene region (section of a mitochondrial DNA cytochrome c oxidase subunit I, COI) against a DNA database (Smith et al., 2008; Silva et al., 2011a). Steinke et al. (2009) examined the COI sequences of 391 species of fish traded for marine aquariums. These authors found that 98% of studied species display sequences that allow their clear separation from any other taxon (including the 6,175 fish species in the Barcode of Life Data System). Thus, this method may be useful to identify ornamentals on the level of species when it is not possible by external characteristics (e.g., cryptic species). Nevertheless, as with most analytical methods, this approach also requires a tissue sample from the target animal, which limits its use on marine ornamentals (Figure 2(II),e). Additionally, this method is also unable to distinguish cultured specimens from wild conspecifics, nor to pinpoint their geographic origin (Olivotto et al., 2011).

4.5.2. Microbiological Barcodes

The profile of bacterial communities associated with aquatic organisms, namely fish, has already been successfully used to determine their origin (Le Nguyen et al., 2008; Smith et al., 2009; Tatsadjieu et al., 2010; Ruamkuson et al., 2011). Bacterial diversity is commonly evaluated by amplifying the 16S rDNA from the bacterial genome through a polymerase chain reaction (PCR) and performing a denaturing gradient gel electrophoresis (DGGE) for bacterial-community "fingerprints" (Tatsadjieu et al., 2010). The PCR product may also be analyzed using the terminal restriction fragment length polymorphism (T-RFLP) technique (Smith et al., 2009). In the specific case of fish,

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these techniques have been successfully used employing samples from fish gills, intestine, skin, and mucus (Le Nguyen et al., 2008; Smith et al., 2009; Tatsadjieu et al., 2010; Ruamkuson et al., 2011). For marine ornamental species, sampling the mucus layer appears to be the most suitable option, as mucus can easily be collected in a non-invasive and non-destructive way (Figure 2(III)). This approach appears to be a promising method for tracing marine ornamentals, as it may allow the identification of the geographical origin of target species and the discrimination between captive-bred, captive-reared, and wild-caught specimens. Additionally, as this method may ultimately allow cultured specimens to be traced to the facility where they were produced (Le Nguyen et al., 2008), it may be used to differentiate producers promoting more sustainable culture practices and add value to their products. This relatively fast and inexpensive approach (Smith et al., 2009; Tatsadjieu et al., 2010) is one of the few traceability tools currently available that does not damage screened specimens (a mandatory feature for any traceability tool to be implemented for marine ornamentals). Most available literature addressed the screening of fish, but Smith et al. (2009) suggested that this method could also be successfully employed for mollusc and crustaceans.

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4.6. *Breeding of Hybrid Specimens*

The culture of specimens exhibiting color patterns or shapes that are not displayed by wild conspecifics appears to be a suitable alternative to distinguish captive-bred animals from other sources (captive reared and wild caught) (Figure 2(IV)) (Olivotto et al., 2011). The hybridization of traded species or the selective breeding of unique color morphs can also increase the variety of products available for traders and commonly add value to the traded product. Currently, there is a great variety of hybrid clownfishes (e.g., ORA®, http://www.orafarm.com/, accessed September 2012), but most cultured species still display their wild morphotype (Olivotto et al., 2011). The culture of hybrids may also be a threat to environmental conservation when native species are cultured, as accidental escapees may eventually cross with wild specimens and eventually cause changes in the pool genic of wild populations with unpredictable impacts to marine ecosystems.

4.7. *Trading New or Rare Species*

According to Olivotto et al. (2011, p. 155), “the aquaculture of species never before traded for marine aquariums may be a potential short-term solution to trace cultured organisms.” The rationale for this approach is as follows. If a species is first introduced in the marine aquarium trade by aquaculture production and wild specimens are not available, all traded animals of this specie will have to be cultured and suppliers may easily be identified. If cultured specimens, however, are identical to wild conspecifics and somehow wild specimens start entering the trade due to an increase in their demand, the traceability of produced specimens will be lost (or at least difficult to achieve). This scenario already occurred with the ornamental shrimp *Lysmata seticaudata*, a unique case study in the marine aquarium trade; this was the first marine ornamental species that was presented to the marine aquarium hobby using cultured specimens before any wild specimens were ever traded. It was only a matter of months until shrimps collected from the wild entered the industry, some of them even being deceptively traded as being cultured in captivity (Calado, 2008). The risks of recruiting new species to the marine aquarium industry have already been highlighted (Tlusty, 2002, 2004; Calado and Dinis, 2008), and the trade of these new species as an attempt to ensure their traceability is likely to work solely for the short term.

5. *CONCLUDING REMARKS*

The amount of illegal, unreported, and unregulated fishing practices associated with the trade of marine ornamental species, together with high levels of mortality along the supply chain, seriously question the sustainability of this activity as it currently stands.

If these issues continue to be overlooked and no proactive measures are implemented, the whole industry is likely to collapse in the years to come. Traceability and certification are therefore important management tools to assist this industry on its path toward environmental, social, and financial sustainability. The trade of marine ornamentals is comparable to no other on its modus operandi: it trades millions of live marine specimens from thousands of species originating from a multitude of locations at a unitary basis, fetching high market values mostly due to their aesthetic appearance. Available methods currently used to trace aquatic animals are not fully suitable to trace marine ornamentals because most of them are invasive, unaesthetic, or require the sacrifice of marked specimens to retrieve the information needed for their identification. These methods may be applied to trace species with low unitary value (e.g., some damselfish, hermit crabs, and snails), as some specimens may be randomly sampled to infer the results for a pool of traded organisms without significant economic losses. It is important to note, however, that such methods violate the principles of animal welfare and should not be encouraged, namely on an industry that commonly advertises marine conservation.

The suitability of all traceability methods addressed in the present work for the trade of marine ornamental species is represented in Figure 3. The use of microbiological barcodes present in the mucus of fish and invertebrates appears to be the best option to trace marine ornamentals given its non-invasive and non-destructive approach along with its high reliability. The breeding of hybrid specimens, as well as certification and eco-labeling were also classified as desirable methods to trace marine ornamentals (Figure 3). Both methods, however, have constraints to trace marine ornamentals in the near future, such as the lack of culture protocols for most traded species, which impairs the

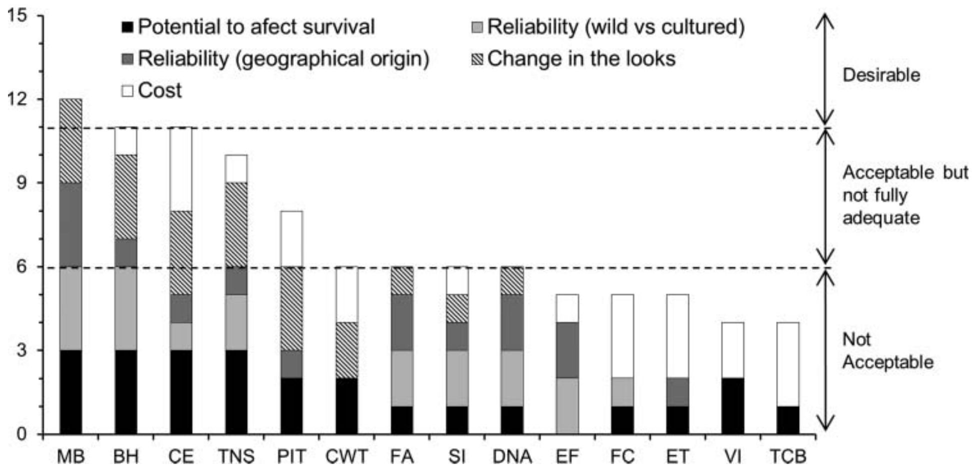

Figure 3 Suitability of traceability methods for the trade of marine ornamental species. Values ranging from 0 to 3 were employed to classify each of the following features: potential to affect survival of the technique (high = 0, none = 3); reliability of the technique (to trace geographical origin) (none = 0, high = 3); reliability of the technique (to discriminate between wild versus cultured specimens) (none = 0, high = 3); cost of the technique (very high = 0, low = 3); change in the looks of target specimens following the application of the technique (high = 0, low = 3) (MB: microbiological barcodes, BH: breeding hybrid specimens, CE: certification and eco-labeling, TNS: trading new or rare species, FA: fatty acids, SI: stable isotopes, DNA: DNA barcodes, EF: elemental fingerprint, FC: fin clipping, ET: external tags, VI: visible implants, TCB: thermal and chemical branding).

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breeding of hybrid specimens, and the high possibility of fraud along the chain of custody, which may threaten the reliability of certification and eco-labeling efforts. Nonetheless, it is important to note that any traceability tool (or tools) employed in this industry must be coupled with a reliable certification program. Without changing the paradigm of the supply chain that has prevailed in the industry in the last decades, any serious attempt to achieve traceability is destined to fail. It is therefore urgent to shift from a long, complex, and fragmented supply chain to a shorter and more integrated model. Moreover, only if marine aquarium keepers become more aware of the sustainability issues associated with the industry that supplies their hobby and are willing to pay more for the added value of certified products will the traceability of marine ornamental species ever be possible.

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