The Effects of Venting and Decompression on Yellow Tang (*Zebrasoma flavescens*) in the Ornamental Aquarium Fish Trade

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Introduction

Each year, over 45 countries remove and export up to 30 million fish from coral reefs as part of the ornamental marine aquarium trade (Bruckner 2005; Wood 2001). Although ~90% of freshwater aquarium fish are successfully cultivated in aquaculture facilities, most tropical marine aquarium fish are wild-caught (Wood 2001). Collecting live fish for the aquarium trade involves removing reef fish from SCUBA diving depths (~10 – 35 m) to the surface, followed by sequentially transporting them from the collection site to an export facility to an import facility to an aquarium fish retail store, and finally, to a hobbyist aquarium. Mortality may occur at any point in this supply chain, impacting each participant in the industry, and negatively affecting coral reefs through increased collection pressure to replace losses (Stevenson et al. 2011; Tissot et al. 2010).

Aquarium fisheries that utilize destructive fishing practices have high mortality. In the Philippines and Indonesia where cyanide is used to stun ornamental fish for ease of capture, >90 % of fish suffer mortality and coral reefs are severely damaged (Hall and Bellwood 1995; Hanawa et al. 1998; Rubec et al. 2001; Rubec and Cruz 2005; Jones and Hoegh-Guldberg 1999; Jones and Steven, 1997). While fishers in Hawaii do not use cyanide to collect fish, and immediate mortality is low (<1%) (Stevenson et al. 2011), it is possible that fishers' collection methods result in delayed mortality. Because fish move rapidly through the supply chain, it is possible that aquarium fishers are unaware of collection methods that result in mortality further along the supply chain. Economically, delayed mortality shifts the burden of fish death and monetary loss from the collector to those further along the supply chain (e.g. the importer, or hobbyist) while also increasing the demand for fish and exacerbating pressure on coral reef ecosystems. Identifying methods that cause delayed mortality would reduce the overall mortality of aquarium fish in the aquarium trade, and thus the number of fish removed from the reef to compensate for these losses.

In order to identify industry methods that cause delayed mortality in aquarium fish, it is necessary to examine each link in the aquarium fish trade supply chain both independently and in succession. Here, we begin by examining the very first step involved in the supply chain: removing fish from depth to the surface. Mortality caused by removing live fish from coral reef depths to the surface is an important and controversial issue affecting this fishery, and ours is the first study to address this problem.

To ensure that fish survive the transition from depth to the surface, aquarium fishers must either prevent or mitigate barotrauma. Fish experience barotrauma because when they are brought to the surface the water pressure decreases, resulting in an increase in the volume of swim bladder gases. This phenomenon is a result of Boyle's Law, in which decreasing pressure causes an exponential increase in gas volume. Barotrauma signs in fish manifest both externally and internally and include: positive buoyancy caused by overexpansion of the swim bladder; bulging of the eyes, or exophthalmia; and protrusion of the intestine from the cloaca. While barotrauma has not been studied in shallow-dwelling (15-18 m) reef fish caught for the aquarium trade, there is ample research on the effects of depth changes on deeper dwelling (20-152 m) fish caught commercially and recreationally for human food consumption.

Research on deeper dwelling food fishes has revealed that protrusion of the esophagus from the mouth is common (Parker et al. 2006; Pribyl 2010; Wilde 2009). In addition, internal signs of barotrauma such as swim bladder rupture, internal bleeding, compression of and damage to organs surrounding the swim bladder, stretching of optic nerves, emphysema of the heart ventricle and epithelial surfaces, and gas emboli in the rete mirabile and kidney caused by gas leakage from the swim bladder (Gotshall 1964; Bruesewitz et al. 1993; Parker et al. 2006; Rogers et al. 2008; Pribyl 2010).

Prior research has also demonstrated that though external signs of barotrauma subside in the short-term, fish continue to exhibit internal injuries for extended periods. Pribyl (2010) found that sublethal effects (not having caused death) from barotrauma such as rupture of the outer layer of the swim bladder (tunica externa) persisted for at least one month after collection in rockfish (genus *Sebastes*). In addition, Hannah and Matteson (2007) determined that barotrauma could reduce post-release survival of fish through behavioral impairment. These findings indicate that sublethal signs of barotrauma persist long after the initial trauma occurs. Knowing this, we predict that fish collected for the live ornamental aquarium trade also suffer sublethal injuries that remain undetected. If infections occur, these sublethal injuries could result in delayed mortality of aquarium fish.

Because barotrauma can be potentially fatal to both shallower-dwelling aquarium fish and deeper-dwelling food fish alike, fishers implement methods that either prevent or mitigate it. Venting is a method that mitigates barotrauma and involves puncturing a fish swim bladder with a hypodermic needle to allow expanded gases to escape, relieving positive buoyancy. Decompression, in contrast, is a method that prevents barotrauma. Decompression involves transporting fish from depth to the surface over a longer period of time, which allows expanding gases to be removed from the swim bladder, resulting in a fish that is not subjected to barotrauma at all. Fishers implement one, or some combination of both of these methods in order to help fish survive the pressure transition. While the use of venting and decompression on aquarium fish has been documented (Randall 1987; Pyle 1993; LeGore et al. 2005), ours is the first study to evaluate the efficacy of each of these procedures in preventing mortality. While the effects of venting and decompression on aquarium fish has not been documented, these methods have been fairly well studied in deeper-dwelling food fishes.

In these deeper-dwelling fishes, decompression takes a long time – up to several days – which is a direct result of the depths these fish are removed from (Parker et al. 2006; Pribyl 2010). Decompression is a time-consuming process because in order to prevent barotrauma, one must allow adequate time for fish to naturally remove the expanding swim bladder. Likewise, in Hawaii, decompression can be prohibitively time-consuming for fishers to implement; even for shallow-dwelling reef fish. The time-consuming nature of decompression deters fishers who would rather remove fish quickly from depth so as to return to depth and collect more fish. However, bringing fish up to the surface quickly without decompression stops results in barotrauma. To mitigate barotrauma, fishers use venting.

Studies on deeper-dwelling food fishes do not definitively conclude that venting actually reduces fish mortality. However, this is largely an artifact of the great differences in species and depths that the studies examine (Gotshall 1964; Keniry et al. 1996; Collins et al. 1999; Kerr 2001; Nguyen et al. 2009; Wilde 2009). In addition, differences in the length of time fish are observed in captivity following removal from depth causes conflicting results (Keniry et al. 1996). This suggests that longer-term holding will allow for specific conclusions about the collection methods employed by fishers. With this in mind, we are careful to employ an experimental design that incorporates both short-term observations and long-term holding.

As previously stated, fishers often use some combination of decompression and venting. For example, it is common practice for aquarium fishers to perform one or several decompression stops, pausing in the water column at intermediate depths before removal to the surface (LeGore et al. 2005; Stevenson et al. 2011). In Hawaii, fishers typically vent the fish following this practice.

These methods of barotrauma prevention and mitigation not only affect fish health and mortality, but are also controversial among the animal rights community. In Hawaii, such groups have repeatedly proposed legislation that would ban the harvest of marine species for the aquarium trade based on animal cruelty claims (i.e. Lauer 2011; Talbot 2012; Wintner 2010, 2011). Groups opposed to venting claim that it inflicts stress and mortality on fish, while collectors maintain that venting is necessary for fish survival. People who oppose venting have suggested that decompression be used instead. While we may not solve the values conflicts driving in this controversy, we do hope to inform pending management decisions related to aquarium fish collection in Hawaii.

In our study, we seek to: (1) Determine short- and long-term mortality of reef fish caught for the aquarium trade subjected to the barotrauma prevention and/or mitigation practices of decompression and venting, respectively; (2) Examine sublethal effects of collection that could result in delayed mortality.

Methods

Experimental Design

The Yellow Tang (*Z. flavescens*) was selected as the study animal because it is the most commonly targeted aquarium species in West Hawaii, consistently composing nearly 80% of the total catch of aquarium fish there (Cesar et al. 2002; Tissot and Hallacher 2003; Walsh et al. 2004; Williams et al. 2009). Therefore, understanding how collection practices affect Yellow

Tang health and survival is especially relevant to the West Hawaii aquarium fishery. In addition, Acanthuridae, the family encompassing Yellow Tang and other surgeonfishes, is one of the most common families targeted globally in the live aquarium trade (Rhyne et al., 2012).

This work was performed under WSU IACUC protocol #04151-004. To examine short- and long-term mortality of ornamental aquarium fish as it relates to collection practices, Yellow Tang were subjected to different collection methods and subsequently held for 21 days (d) for observation at an aquaculture facility in West Hawaii. Fish suffering mortality were examined histologically to identify lesions that could have contributed to death. A subset of fish surviving the holding period were also histologically examined. Serum cortisol concentration was measured because it can serve as a proxy for stress in fish (Donaldson 1981).

A fully crossed factorial experimental design was used, with three decompression treatments, coupled with or without venting in all possible combinations (k=6 treatments). Each treatment was replicated three times, with n=20 fish in each treatment combination for a total of 360 individuals. A subset of fish (n=5) was sacrificed immediately following collection in each treatment replicate for histopathology and to assess post-collection cortisol. Fish were collected between 15-18 m depth, reflecting the range frequented by West Hawaii collectors (Stevenson et al. 2011). In order to accurately reflect methods used by aquarium fishers, an experienced aquarium fisher performed fish collection. Fish collection occurred on SCUBA using a barrier net, as described by Stevenson et al. (2011). When the desired quantity of fish (n=40) was caught, they were transferred to containers assigned to each ascent treatment. Following ascent to the surface vessel, half (n=20) of the fish were vented treatment and half were not.

Three decompression treatments were used: 1) ascent without decompression stops, 2) ascent with one decompression stop, and 3) ascent with multiple decompression stops. The rate of ascent between decompression stops was 0.25 $m \cdot s^{-1}$ for all treatments, the recommended SCUBA ascent rate and the rate fishers ascend while transporting fish from depth to the surface. Fish subjected to ascent without decompression were brought directly to the surface from depth. Fish subjected to ascent with one decompression stop were brought up to half the maximum depth for a 45 min decompression stop, and then brought to the surface. Fish subjected to multiple decompression stops were brought up 3 m every 15 min and at 10 m (2 atm), these fish were brought up 1.5 m every 15 min because the volumetric change resulting from the decrease in pressure is especially great the last few meters of ascent.

As is typical in the fishery, venting was performed by the fisher on the fishing vessel using a 20 G hypodermic needle, replaced after approximately 50 fish. Each fish was held out of water for <3 s by the fisher while the needle was inserted through the body wall toward the swim bladder, caudal to the pectoral fin and ventral of the lateral line.

During transport, each replicate group was held separately in the collector's live well. During collection and transit from collection site to port, the water in the live well was continuously exchanged with fresh seawater.

Holding Period

Post-collection, fish were observed for 21 d at an aquaculture facility located at the Natural Energy Laboratory Hawaii Authority in West Hawaii provided with natural surface seawater at ambient temperatures. The experiment duration was chosen because after interviewing West Hawaii fishers, it was determined that 21d represents a reasonable time period for a fish to be transferred from the reef to a retailer or hobbyist in this particular supply chain. In addition, swim bladder healing in rockfish has been observed after 21 d (Parker et al. 2006) and is sufficient time to allow skin and muscle regeneration in fish (Roberts 2010). Therefore, fish exhibiting lesions after 21 d may not have fully recovered in a supply chain environment and could be categorized as having sublethal effects from collection.

Fish were held in 1 m diameter mesh floating cages within three 10,000 l pools, which served as replicate blocks, each containing all six treatments. Incoming seawater was filtered to 5µm, and set to flow through each pool at a rate of 1 volume□d^{-1}. Pools were exposed to natural sunlight, and temperatures was measured twice daily.

All fish were fed a natural algae diet (*Ulva fasciata*) rich in nutrients (primarily nitrogen) absorbed from food fish outflow in the aquaculture facility. Aquaculture facilities use algae such as *Ulva spp*. for biofiltration (Vandermeulen and Gordin 1990; Jiménez del Río et al. 1996). The algae accumulates nutrients and can serve as a nutrient rich food source for herbivorous fish like Yellow Tang.

Fish were monitored daily and mortality was recorded. Standard length (SL) (from snout to base of caudal fin) of each fish was measured. Following mortality, fish were placed in 10% neutral buffered formalin for histopathology; the operculum was removed and body cavity opened to facilitate proper formalin fixation of the internal tissues. Moribund fish were humanely euthanized using an overdose solution (> 250 mg□l^{-1}) of tricaine methanesulfonate (MS-222).

Histopathology

To determine the sublethal effects of collection methods, fish (n=5) were chosen randomly from each replicate treatment group immediately upon arrival to the holding facility (0 d) and at the end of the holding period (21 d) for histopathology. Fish used for histopathology were euthanized using an overdose solution of MS-222, placed on ice, and shipped within 48 h to Oregon State University's (OSU) Veterinary Diagnostic Laboratory (VDL) for histologic examination. Fish that died during the experiment were fixed in 10% neutral buffer formalin as described above and examined.

Formalin-fixed fish were immersed for 24 h in Cal-Ex II (Fisher Scientific) to decalcify bone, and serial cross sections were processed using standard histologic techniques, sectioned at 5 μm, and stained with hematoxylin and eosin. Brown-Hopps Gram stain was used as necessary to assess for the presence of bacteria. All slides were examined using a Nikon Eclipse 50i microscope. Histologic examination focused upon gill, heart, kidney, liver, swim bladder, and intestine.

Primary Stress Response

Because of the potential for cortisol concentrations to decrease when a stressor subsides, blood samples were collected from fish immediately upon arrival to the holding facility. Fish (n=2) were anesthetized from each treatment replicate group using MS-222 prior to drawing 0.3-1.0 ml blood from the heart using a 25G 2.54 cm needle and 3 ml syringe. Cardiac puncture was necessary because the small size of the fish. Following blood sample collection, fish were euthanized using an overdose solution of MS-222. To determine Yellow Tang ocean baseline cortisol concentration, blood was collected from fish (n=4) underwater on SCUBA at capture depth within 3 min of capture. Blood was injected into 3 ml vacutainer tubes with no additive (Becton-Dickinson), placed on ice, and centrifuged at 3,000 rpm for 10 min <1 h later. Serum supernatant was transferred to a clean vacutainer tube with no additive, placed on ice, and frozen

<1 h later for ≤ 40 d in a non-frostless freezer, and transported overnight on dry ice to the OSU Department of Fisheries and Wildlife for analysis.

Serum cortisol concentrations were determined using radioimmunoassay (RIA) as described by Redding et al. (1984). Total binding, the ratio of the radiolabeled cortisol bound to the antibody to the total amount of radiolabeled cortisol in the sample, was 40-50%. Samples showed adequate parallelism, and 3.9-500.0 ng•ml^{-1} cortisol standards were used.

Statistical Methods

Statistical analyses were performed using the Minitab 15 Statistical Software program. To meet assumptions of normality and homogeneity of variance, data were transformed to square root (fish SL) or log (cortisol). A one-way t-test was used to compare mean cortisol concentrations of each treatment group with the ocean baseline parameter. A two-way ANOVA was used to compare mean cortisol concentrations, with decompression treatment and venting as fixed factors and replicate block as a random factor. Tukey's multiple comparisons test was used to determine significant differences between levels within each factor.

Results

Mortality

Sizes of Yellow Tang in this study ranged from 5.0-10.0 cm SL with a mean value of 7.2 cm (*SE*=0.05 cm). Mortality occurred <24 h post-collection in fish subjected to ascent without decompression stops or venting, with a mean mortality of 6.2% (*SE*=0.6%). No mortality occurred in the other experimental treatments.

The incidence of mortality was consistent with observations of the frequency and severity of external barotrauma signs. These included high frequency of positive buoyancy, bloating, prolapse of the intestine from the cloaca (Figure 1), and exophthalmia in fish subjected to ascent without decompression stops. Venting relieved positive buoyancy and vented fish became neutrally or negatively buoyant (Figure 1).

Histopathology

Histopathology of gill, heart, kidney, liver, swim bladder, and intestine failed to detect significant inflammation, necrosis, or gas embolism associated with barotrauma or venting in any treatment, in both the short- and long-term. A venting wound was detected in a fish subjected to ascent with many decompression stops and venting sampled immediately after collection. However, this lesion consisted only of locally extensive necrosis of body wall musculature and a localized influx of neutrophils surrounding the needle track and not significant widespread infection (Figure 2).

Primary Stress Response

The mean ocean baseline cortisol concentration was 8.9 ng•ml^{-1} (*SE*= 4.96 ng•ml^{-1}) and in some cases was at or below the detection limit for the assay (3.9 ng•ml^{-1}). All treatment groups were significantly elevated above the baseline cortisol concentration (all $p < 0.05$). Decompression treatment significantly affected cortisol concentration (Two-way ANOVA: F=4.26; df= 2,10; p=0.03). Ascent without decompression stops resulted in a significantly higher mean cortisol concentration (M=58.8 ng•ml^{-1}, SE=8.7 ng•ml^{-1}) than ascent with many 15 min decompression stops (M=35.5 ng•ml^{-1}, SE=5.3 ng•ml^{-1}), with neither treatment being significantly different from ascent with one 45 min decompression stop (M=35.2 ng•ml^{-1}, SE=4.3 ng•ml^{-1}) (Figure 3).

Ascent without decompression stops produced the highest observed cortisol concentration (101.49 ng•ml^{-1}), whereas the highest observed cortisol concentrations in fish subjected to one and many decompression stops were 59.09 and 68.03 ng•ml^{-1}, respectively. While venting resulted in higher mean cortisol concentration (M=47.7 ng•ml^{-1}, SE=6.9 ng•ml^{-1}) than the no venting treatment (M=38.2 ng•ml^{-1}, SE=4.3 ng•ml^{-1}), this difference was not statistically significant. In addition, there was no significant interaction between decompression treatment and venting.

Discussion

With the objective of informing management on collection practices in the aquarium trade, our study focused on the short- and long-term mortality of reef fish subjected to decompression and venting as barotrauma prevention and mitigation practices, respectively. Overall, we found that venting prevented immediate mortality in fish subjected to ascent without decompression stops. Furthermore, we found only one case of a venting needle track, and the inflammation was localized. There was no evidence of significant widespread tissue inflammation caused by venting, or lesions linked to barotrauma immediately after collection, or following the long-term 21 d holding period. Finally, ascent to the surface significantly elevated serum cortisol above baseline concentrations in fish at depth. Ascent without decompression stops resulted in significantly higher serum cortisol concentrations than ascent with many stops. Venting did not significantly affect cortisol concentrations, nor were there any significant interactions between decompression and venting. In the following sections, we explain our results, suggest future research recommendations, and discuss implications for fishery management.

Mortality

We found that the methods commonly used in this fishery (ascent without decompression stops, or ascent with one decompression stop, followed by venting) resulted in no immediate or delayed mortality. Ascent without decompression stops followed by venting resulted in no mortality, while fish subjected to ascent without decompression stops and no venting was the only treatment group to suffer mortality. Venting alleviated positive buoyancy in fish following ascent with no decompression stops and in this way mitigated barotrauma sufficiently to prevent short-term mortality. Neutral buoyancy allowed fish to control body position and avoid colliding with the transport container during transport from reef to harbor. This is in contrast to fish subjected to ascent without decompression or venting, which exhibited positive buoyancy and were at risk of acquiring secondary transport-related injuries.

Additional factors that may influence post-collection mortality, but are outside the scope of this study, include collection depth, body size, and species. We examined fish collected from 15-18 m depths, which is typical for the West Hawaii Yellow Tang fishery, though fishers do exceed this range (i.e. ≥27 m) when targeting other species (Stevenson et al. 2011). At deeper depths, the effects of decompression and venting may differ, and it is known that fish mortality and occurrence of barotrauma increases with capture depth (Collins et al. 1999; St John and Seyers 2005; Hannah et al. 2008; Jarvis and Lowe, 2008; Campbell et al. 2010). Interviews with West Hawaii fishers indicate that fish collected from >25 m require more decompression time and venting while at depth, or several venting applications during ascent. Fishers have also mentioned that larger fish exhibit more severe external barotrauma symptoms than smaller fish of the same species, which is similar to findings in studies on deeper-dwelling food fishes (Hannah et al. 2008; St John and Seyers 2005). Just as different deeper-dwelling food fish species exhibit different responses to ascent rate (Hannah and Matteson 2007; Jarvis and Lowe

2008; Pribyl 2010), aquarium fish species reportedly react differently to ascent rate and venting. These differences are likely caused by variation in body shape, tissue durability, and swim bladder volume between species. Methods used by fishers reflect these species differences, with practices such as performing venting on more delicate, soft-bodied fish like angelfish (Pomacanthidae) underwater to prevent swim bladder expansion. Examining differences among aquarium fish species of varying sizes and investigating the variety of techniques employed by fishers during collection would provide further insight into the prevalence and effectiveness of aquarium fish barotrauma prevention and mitigation methods.

Histopathology

Histopathology did not detect significant widespread inflammation, organ damage or infection caused by venting. Only one case of a needle wound was found that consisted of localized necrosis and inflammation, with no visible evidence of infection. It is possible that histologic sectioning of tissues missed similar lesions in other fish, but this was minimized by focusing the sampling at the site consistently used by fishers for venting. However, the objective of histopathology in our study was to determine if widespread inflammation or tissue damage was present in fish indicating significant injury, which was not found. If such injuries were present, they would have been detected in multiple sections of the tissues surrounding the venting wound.

Wound healing with no evidence of ongoing necrosis or inflammation, as seen in these fish, indicates that the venting procedure does not pose a significant threat to fish survival post-collection, nor does it cause significant sublethal effects. However, we caution that the fish in our study were held in an aquaculture facility for 21 d without the additional handling and transport stressors they would normally experience in the supply chain, thus potentially promoting recovery from injuries inflicted during collection. It is possible that additional stressors of the supply chain diminish the efficacy of venting in promoting long-term fish survival.

Because aquarium fish exhibited external signs of barotrauma similar to those observed in deeper-dwelling food fishes, we expected internal barotrauma signs to be similar as well. However, we did not detect lesions resulting from barotrauma, even in fish subjected to ascent without decompression. Externally visible signs of barotrauma did occur, however. Positively buoyant fish were bloated and had intestinal prolapse at the cloaca. Although not examined in this study, it is likely that organ displacement by the swim bladder occurred in these fish, an internal barotrauma sign observed in deeper-dwelling food fishes (Rogers et al. 2008). Determining if organ displacement occurs, and if venting relieves this issue in aquarium fish would further our understanding of the mechanisms with which venting reduces mortality in fish subjected to ascent without decompression.

Primary Stress Response

Our results indicate that all collection methods produced elevated cortisol concentrations above the ocean baseline level. Though we did not perform stress treatments on Yellow Tang to determine a cortisol level that corresponds to a stressed state, Soares et al. (2011) did so with a closely related acanthurid (*Ctenochaetus striatus*). While cortisol concentrations vary between species (Barton and Iwama 1991), stressed (45-65 $ng \bullet ml^{-1}$) and non-stressed (10-25 $ng \bullet ml^{-1}$) cortisol concentrations in *C. striatus* suggest that venting increased stress in fish subjected to ascent without decompression though this was not statistically significant. Despite this increase, we emphasize that venting did mitigate positive buoyancy and ultimately prevented mortality. It appears that venting is a short-term stressor, but prevents mortality in fish subjected to ascent without decompression stops.

Future studies should investigate if cortisol levels subside, or remain elevated in the rest of the supply chain. Handling in and transport between export, import, and retail facilities may exacerbate collection-induced stress. Because chronic stress results in immune system suppression (Barton and Iwama 1991; Barton 2002), fish experiencing chronic stress are more susceptible to infection, disease, and delayed mortality. Because hobbyists whose aquarium fish die often replace these fish, delayed mortality is a great driver of aquarium fish demand (Tissot et al. 2010). It is likely that stress plays a role in this mortality, and future studies should examine stress as it relates to handling in and transport between each link in the supply chain beyond collection.

Implications for Management

While our work adds to scientific knowledge regarding collection practices of aquarium fish in Hawaii, it is also relevant to the global trade. Yellow Tang and other surgeonfish (family Acanthuridae), are one of the most common families targeted globally in the live aquarium trade (Rhyne et al., 2012). Our results also improve our understanding of the effects of venting. Previous studies show conflicting results regarding the effects of venting on fish mortality (Gotshall 1964; Keniry et al. 1996; Nguyen et al. 2009; Wilde 2009). Our results indicate that when performed properly, venting does not cause mortality or inflict significant sublethal injuries, though we caution that our inference is limited to a single species.

Though animal rights groups in Hawaii criticize venting, we did not find that it caused mortality or sublethal injuries in Yellow Tang. Banning venting may increase mortality rates if fishers implemented ascent without decompression. While opponents of venting have suggested that slow decompression be used instead, the time required to properly decompress these fish is economically prohibitive and impractical for fishers to implement.

In conclusion, we determined that the methods commonly used by aquarium fishers in Hawaii do not cause mortality in Yellow Tang. However, all collection methods produced elevated cortisol concentrations in fish, and this warrants more investigation. Further handling in and transport between links in the supply chain could cause chronically elevated cortisol concentrations in fish, exacerbating stress and minor injuries inflicted during collection.

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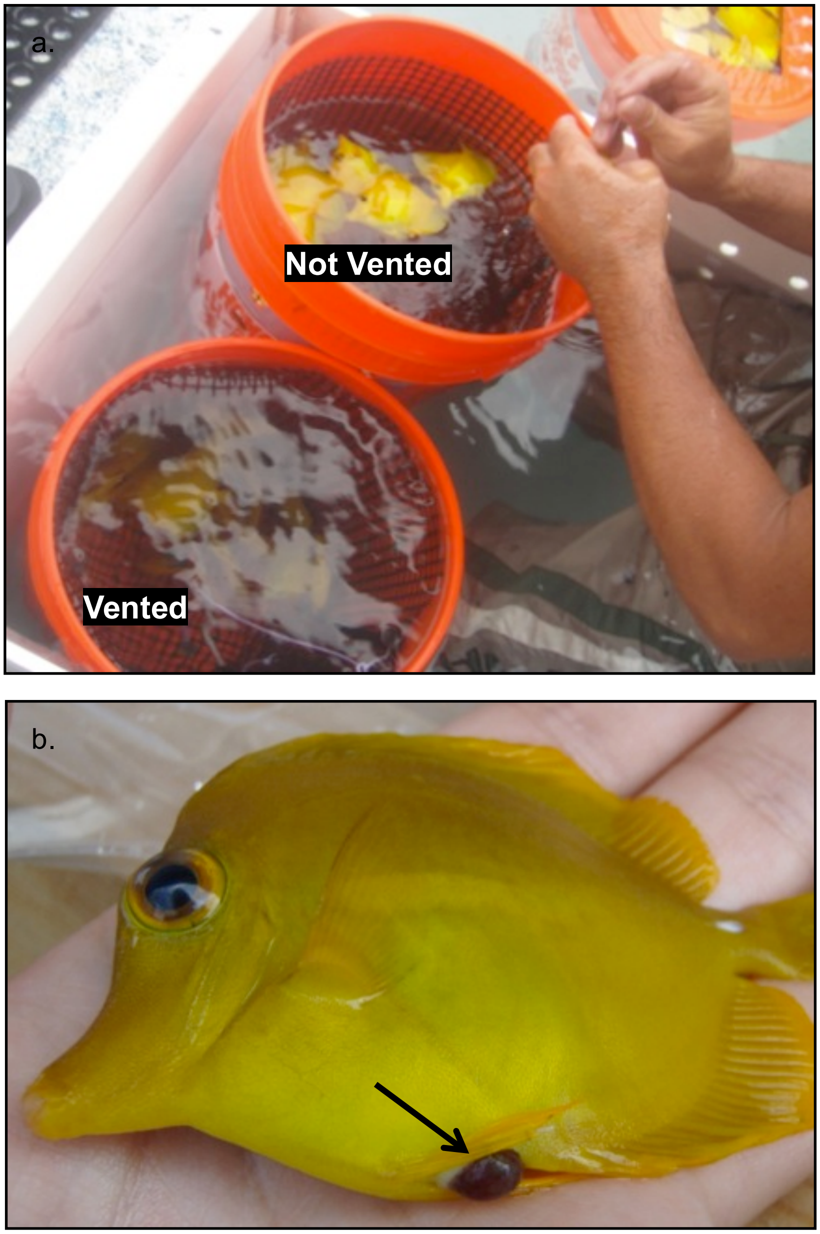

Figure 1: Barotrauma signs observed in Yellow Tang following collection: (a) positive buoyancy before venting and neutral to negative buoyancy following venting (b) intestinal protrusion from the cloaca.

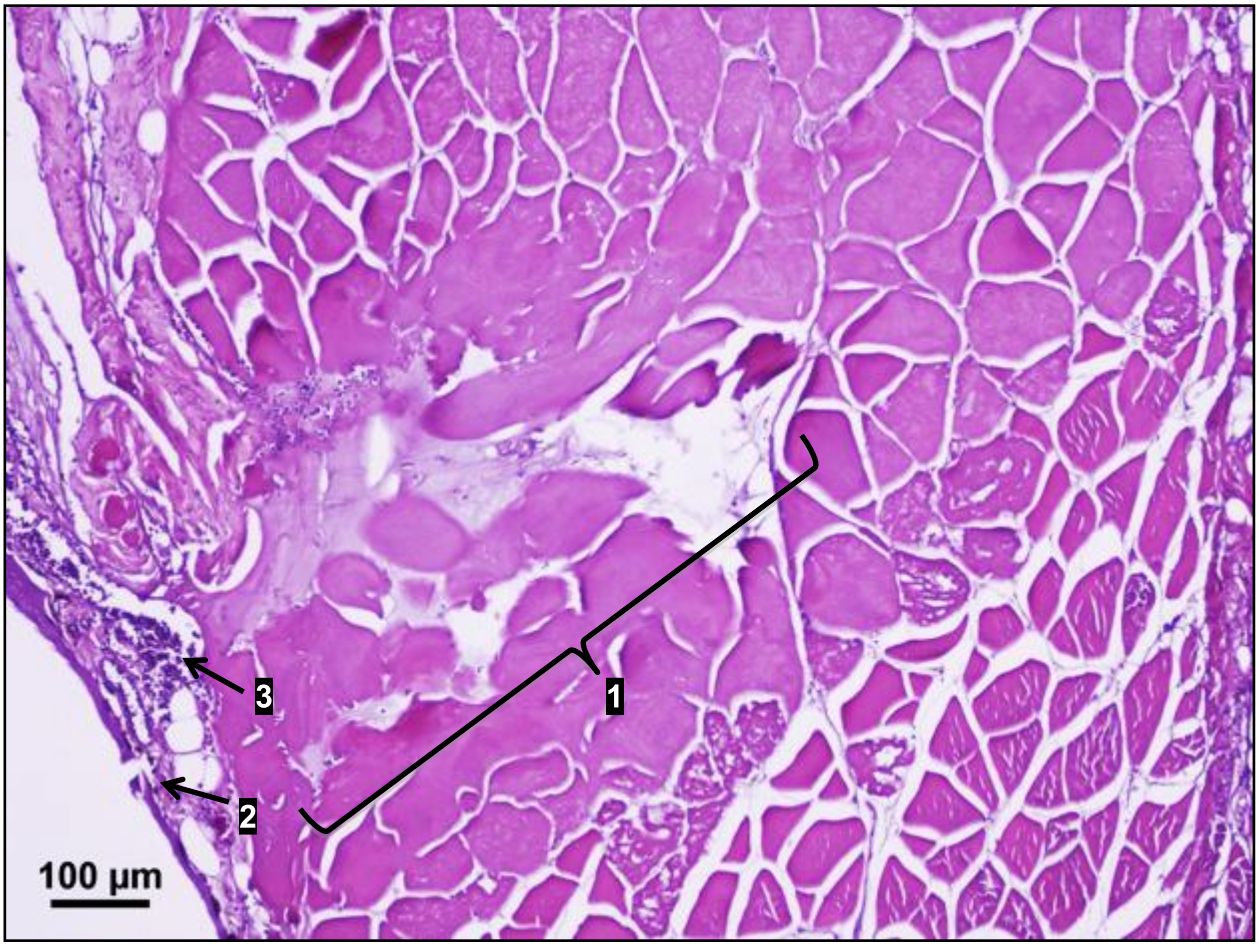

Figure 2: Histological section of needle track in a Yellow Tang subjected to venting showing muscle cell necrosis, edema, and neutrophilic inflammation, at 10x magnification. (1) Needle track, (2) needle entry through coelomic cavity, (3) neutrophilic inflammatory response.

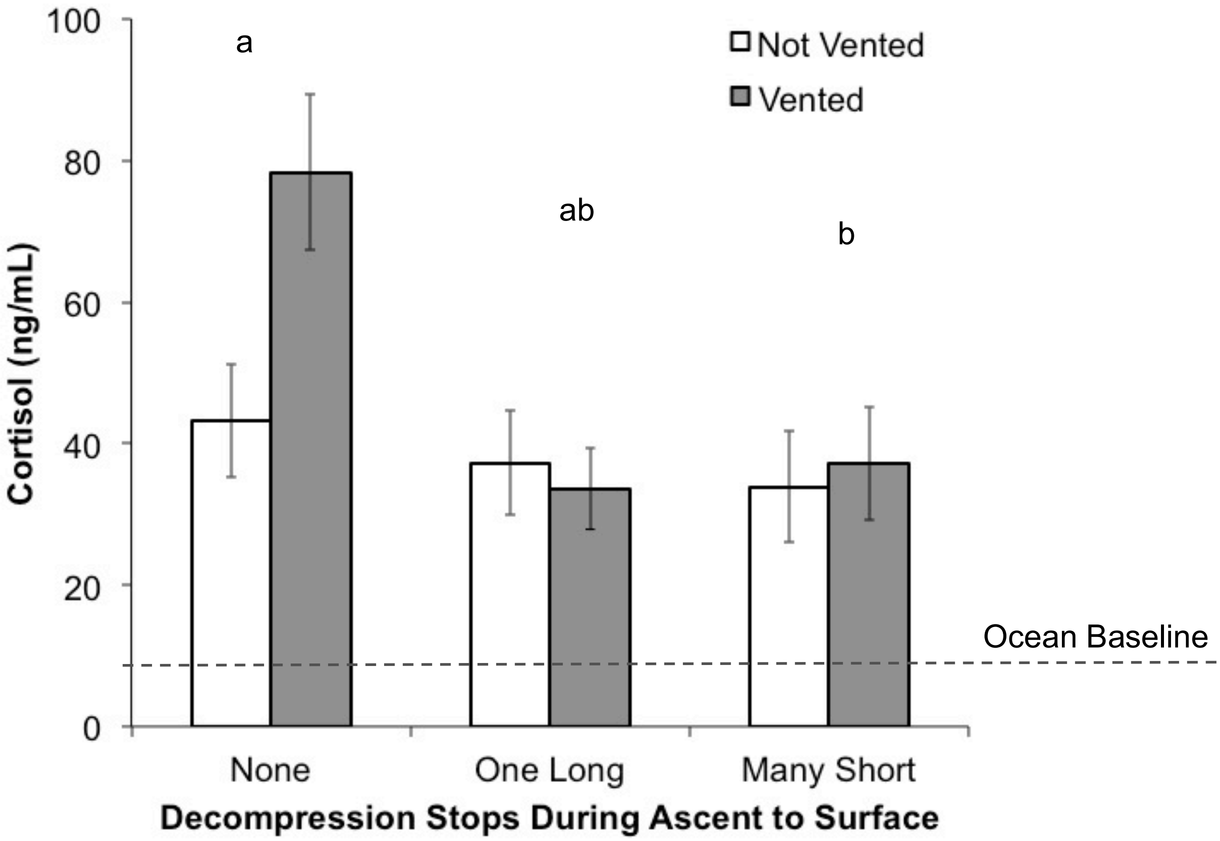

Figure 3: Cortisol concentration (mean +/-SE) by each treatment. Letter groups represent Tukey's multiple range test results comparing means between decompression treatments. All treatment groups were significantly elevated above the ocean baseline concentration of 8.9 ng•ml^{-1}.