THE EFFECTS OF VENTING AND DECOMPRESSION ON MORTALITY AND SUBLETHAL EFFECTS IN YELLOW TANGS (*ZEBRASOMA FLAVESCENS*) CAUGHT FOR THE WEST HAWAII AQUARIUM TRADE

By

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To the faculty of Washington State University:

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Abstract

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Each year, over 45 countries export 30 million fish from coral reefs as part of the global marine ornamental aquarium trade. Most fish harvested in the United States are collected in West Hawaii, where aquarium fishers annually remove over 400,000 fish from coral reefs. Recent discussions surrounding this fishery have centered on managing fish collection methods to reduce this catch volume. We conducted the first comprehensive study on the effects of aquarium fish barotrauma prevention and mitigation practices. Clinical signs of barotrauma caused by a forced ascent from depth can be prevented with decompression, or mitigated with venting (puncturing the swim bladder to release expanded internal gas). We studied the effects of three decompression treatments (fast, intermediate, slow) coupled with, or without venting in a fully crossed orthogonal design on yellow tang (*Zebrasoma flavescens*) mortality and sublethal effects, as elucidated through histology and serum cortisol. In *Z. flavescens*, post-collection mortality of 6% occurred within 24 hours of capture in fish subjected to fast decompression with no venting. The most popular methods in the fishery, fast or intermediate decompression followed by venting, resulted in no mortality. Histopathology of the heart, liver, head kidney,

swim bladder and surrounding tissues in fish sampled 0 and 21 days post-collection revealed no significant inflammation or other lesions in any treatment groups. Fast decompression resulted in significantly higher serum cortisol than slow decompression, and venting alone did not significantly affect cortisol. Future studies should examine the links in the supply chain following collection to determine if further handling and transport stressors affect survivorship and sublethal effects.

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This thesis is dedicated to the yellow tangs of West Hawaii.

THE EFFECTS OF VENTING AND DECOMPRESSION ON MORTALITY AND SUBLETHAL EFFECTS IN YELLOW TANGS (*ZEBRASOMA FLAVESCENS*) CAUGHT FOR THE WEST HAWAII AQUARIUM TRADE

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) and their removal can have negative effects on coral reefs (Tissot et al., 2010). Mortality that occurs after live fish are removed from depth as part of the collection process is one issue that affects the impact of this fishery on aquarium fish populations. Although prior studies have examined the response of deep-water (20-152 m) fish to forced removal from depth, no studies have investigated the effects on shallow (15-18 m) tropical reef fish collected for the live ornamental aquarium trade. This is an important issue because mortality is one driver of the demand for aquarium fish, in which more fish are removed from the reef to replace losses (Stevenson, et al. 2011). Live fish collection for the aquarium trade involves removal of reef fish from depth to the surface while transport encompasses the movement of collected fish to an export facility, where they are held prior to being shipped to an importer, and finally to an aquarium retail store where a consumer can purchase the fish for their home aquarium.

An important yet understudied component of live fish collection is the removal of fish from depth, subsequent transport to the surface, and the methods that must be implemented for fish to survive the pressure transition. As fish are brought to the surface, decreasing external

pressure can result in barotrauma. Barotrauma signs in fish manifest both externally and internally and may include positive buoyancy (bloating caused by overexpansion of the swim bladder), bulging of the eyes (exophthalmia) and protrusion of the intestines from the cloaca. In deep water fish, extrusion of the esophagus from the mouth is common (Parker et al., 2006; Pribyl, 2010; Wilde, 2009). In addition, internal symptoms have been observed in deep water temperate fish which include swim bladder rupture, internal bleeding, compression damage to and displacement of organs surrounding the swim bladder, stretching of optic nerves, emphysema of the heart ventricle, and gas emboli in the rete mirabile and kidney caused by gas leaking from the swim bladder (Gotshall, 1964; Bruesewitz et al., 1993; Parker et al., 2006; Rogers et al., 2008; Pribyl, 2010).

Pribyl (2010) found that sublethal effects from barotrauma-related injuries 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*) without causing mortality. 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 may persist long after barotrauma occurs, and suggest that fish intended for the live ornamental trade may suffer sublethal effects that remain undetected.

Venting and decompression are two common methods that mitigate or prevent barotrauma, respectively, in both deep water food fishes and aquarium fishes. Though the use of these procedures in aquarium fisheries has been documented (Randall, 1987; Pyle, 1993; LeGore et al., 2005), the efficacy of each of these procedures in preventing aquarium fish mortality and sublethal effects has not been evaluated. Decompression at a rate that is natural to fish is determined by the fish's ability to remain neutrally buoyant in the water column while being

decompressed. In deep-water temperate fish, natural decompression can take days (Parker et al. 2006; Pribyl, 2010). Performing one or several decompression stops (pause in the water column at an intermediate depth before removal to the surface) is a technique implemented in aquarium fisheries such as those in Puerto Rico and West Hawaii (LeGore et al., 2005; Stevenson et al., 2011), though its effectiveness at preventing mortality has not been tested. Decompression can be a more time-consuming process that allows fish adequate time to naturally remove the greater volume of air in its swim bladder, thus preventing barotrauma. In contrast, venting allows for fish to be brought rapidly to the surface by puncturing the swim bladder with a hypodermic needle to release excess air in the swim bladder, thus mitigating barotrauma, but potentially causing organ and tissue damage.

Studies on deep-water food fisheries show conflicting results on venting as an effective practice to mitigate barotrauma and increase fish survival (Gotshall, 1964; Keniry et al., 1996; Collins, et al., 1999; Kerr, 2001; Nguyen et al., 2009; Wilde, 2009). Different depth ranges appear to present different survival rates among fish treated with venting (Gotshall, 1964; Collins, et al., 1999; Wilde, 2009), though it is difficult to determine a strong depth pattern associated with venting mortality because few studies have closely examined this interaction, which may also be species-specific. The outcomes of these studies also appear to be based on whether short- or long-term fish survival is examined (Keniry et al., 1996), which is a product of the experimental design: either short-term holding, or long-term mark and recapture. We expect that long-term captive observation of fish post-collection will resolve this issue.

Recently proposed legislation in Hawaii reflects the attempts of a local animal rights group to ban the harvest of marine species for the aquarium trade based on animal cruelty claims and concerns about post-collection mortality (Lauer, 2011; Talbot, 2012a; Wintner 2010, 2011).

Venting is popular among aquarium fishers, but is a disputed collection method in West Hawaii. Opponents claim venting inflicts suffering and mortality on fish, while collectors maintain that venting benefits fish brought up from depth and is necessary for fish survival. Those opposing venting have suggested that slow decompression be used in its stead. This controversy highlights the paucity of data surrounding collection practices in the aquarium industry, not only in Hawaii, but globally. Thus, further research on mortality as it relates to collection is warranted. Legislation should be based on sound science, which should also lay the groundwork for best management practices that are both ecologically sustainable and economically practical.

Although aquarium fisheries where destructive fishing practices like cyanide are used may have high rates (≥90%) of fish mortality (Rubec & Cruz, 2005), Stevenson et al. (2011) estimated the short-term mortality rate of fish collected in West Hawaii as <1% (from collection to harbor). Although short-term mortality is low, it is possible that delayed mortality occurs as a result of sublethal effects and remains undetected in the long-term. Because of the rapid movement of fish through the supply chain, fishers and exporters in West Hawaii may be unaware of specific connections between collection or handling methods and mortality further down the supply chain. Moreover, delayed mortality could shift the burden of fish death and economic loss from the collector to an exporter, or to the importer, retailer, or hobbyist. Importantly, improvements in the collection and transport methods for fish could potentially reduce overall mortality of aquarium fish, thus reducing the numbers of fish that must be removed from the reef to compensate for losses in various stages of the supply chain.

Given the conflict surrounding collection practices in Hawaii and the paucity of data regarding barotrauma mitigation practices in the aquarium trade, it is clear that a scientific study

is needed to investigate the practices used by fishers, while providing recommendations for best management practices. To meet these goals, we address the following objectives:

1. *Determine short- and long-term mortality of reef fish caught for the aquarium trade subjected to barotrauma prevention and/or mitigation practices: decompression and venting, respectively.*
2. *Examine sublethal effects of collection that could result in delayed mortality: gross and histologic lesions, and stress.*

METHODS

Experimental Design

The yellow tang, *Zebrasoma flavescens,* was selected as the study animal because it is the most common species targeted by aquarium fishing in West Hawaii, making up between 65-80% of the total catch of aquarium fish (Cesar, et al., 2002; Tissot & Hallacher, 2003; Walsh et al., 2004; Williams, et al., 2009). Therefore, understanding how collection practices affect yellow tang survivorship and health is especially relevant to the West Hawaii aquarium fishery.

To examine short- and long-term mortality in *Z. flavescens,* fish were subjected to six different collection methods and subsequently held for 21 days (d) for observation. Fish suffering mortality were histopathologically examined to identify specific causes of death. A subset of surviving fish were also examined to determine if sublethal injuries at the tissue level were present. We also measured serum cortisol concentration, a proxy for stress in fish (Donaldson, 1981).

A fully crossed factorial experiment was conducted, with three levels of decompression treatment (fast, intermediate, slow), and two levels of venting (no, yes) 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. Fish were subsampled (n=5) immediately from each of the treatment replicates following collection to establish a baseline for histopathology, and to assess post-collection cortisol levels, resulting in n=15 fish per treatment replicate for subsequent mortality observations. The fish were collected within the depth range of 15-18 m, reflecting the range of depths typically utilized by collectors in West Hawaii (Stevenson et al., 2011). Fish collection was performed by a licensed aquarium fisher using SCUBA with typical methods as described by Stevenson et al. (2011). When the desired quantity of fish was caught, fish were transferred by the collector to containers assigned to each decompression treatment. Following the decompression treatment one half were subjected to the venting treatment and the other half were not.

Decompression procedures are outlined in detail in Appendix Table 1. The fast decompression treatment involved bringing the fish directly to the surface from 15-18 m depths at a rate of 0.25 ms^{-1} (recommended SCUBA ascent rate) resulting in ~1 min total decompression time. In the intermediate decompression treatment, a collection method also utilized by West Hawaii fishers, the collection container was hung off the boat and brought up at a rate of 0.25 ms^{-1} to 6m, half the maximum depth. The fish were then allowed to decompress for 45 min before being brought to the surface. In the slow decompression treatment, which is implemented by West Hawaii fishers on shallower reefs (10-12 m) and on fish species especially sensitive to depth changes, fish were brought up 3 m every 15 min until the collection container was at 10 m (2 atm), then 1.5 m every 15 min. The fast decompression + venting treatment is a method applied frequently by fishers on yellow tangs in West Hawaii, and 100% of fishers interviewed reported using venting, ranging from occasionally to always.

Venting was performed by the fisher on the surface vessel using a 20 G hypodermic needle, replaced after ~50 fish. Each fish was held out of water for ~3-5 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.

Fish were collected over a period of two days. For each collection day, the duration of transport from the collection site to port (<1h) and from port to the holding facility was similar (<1h) to reduce uncertainty associated with differences in fish transport time. During transport, each replicate treatment group was held separately in the collector's live well. During collection and while in transit from the collection site to port, the water in the live well was constantly exchanged with fresh seawater.

Holding Period

Post-collection, fish were observed for 21 days (d) at an aquaculture facility located at the Natural Energy Laboratory Hawaii Authority (NELHA) in West Hawaii provided with fresh surface seawater. The experiment duration was chosen because it represents the approximate time it would take for a fish to be transferred from the reef to a retailer or hobbyist in the aquarium supply chain and because 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).

Fish were held in 1 m diameter mesh floating cages within three 4 m diameter pools filled with ~10,000 l seawater. Each pool served as a replicate block and held all six treatments. Incoming seawater was filtered to 5µm, and set to flow through each pool at a rate of 1 vol. d^{-1}. If parasites were detected on fish, pool water salinity was slowly lowered to hyposaline

conditions (30-14 º/ºº). The pools were exposed to natural sunlight, and temperature was monitored twice daily to record the minimum and maximum temperatures (Appendix Table 2).

All fish were fed a natural algae diet (*Ulva fasciata*) that was rich in nutrients (primarily nitrogen) absorbed from a food fish outflow tank in the aquaculture facility. Aquaculture facilities use algae such as *Ulva spp*. for biofiltration (Vandermeulen and Gordin, 1990; Jiménez del Río, et al., 1996).

Fish health was observed, and any mortality was recorded. Each fish's standard length (SL) (from snout to start of caudal fin) was measured. Post-mortality, fish were placed in 10% neutral buffered formalin for histopathology; the operculum was removed and the body cavity opened to facilitate flow of formalin fixative into the tissues. Fish that were moribund were euthanized using an overdose solution (>250 mg/L) of tricaine methanesulfonate (MS-222).

Histopathology

To determine the sublethal effects of decompression and venting treatments, 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 histology. 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). Fish that perished during the experiment were fixed in 10% neutral buffer formalin and sent to the VDL for histopathology.

For histopathology, the formalin-fixed fish were immersed for 24h in Cal-Ex II (Fisher Scientific) to decalcify bone, after which serial cross sections of the fish were placed in plastic cassettes and processed using standard histologic techniques. Paraffin embedded specimens were sectioned at 5µm and stained with hematoxylin and eosin. Brown-Hopps Gram stain was used to

assess for bacterial growth. Using a Nikon Eclipse 50I microscope, tissues routinely examined for evidence of histologic lesions included gill, heart, kidney, liver, swim bladder, and intestine.

Cortisol

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 (0 d). 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 2.54cm needle and 3 mL syringe. Blood was injected into 3mL Benson-Dickinson (BD) vacutainer tubes with no additive, placed on ice, and centrifuged at 3,000 rpm for 10 min <1 h later. The serum supernatant was transferred to a clean 3mL BD vacutainer tube with no additive, placed on ice, and frozen <1 h later for ≤ 40 d in a non-frostless freezer, and shipped overnight on dry ice to the Schreck laboratory at OSU's Department of Fisheries and Wildlife.

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%. The samples showed adequate parallelism, and 3.9-500.0 ng/ml cortisol standards were used.

To determine the ocean baseline cortisol concentration for *Z. flavescens*, blood was collected from fish (n=4) underwater at depth within 3 minutes of capture. In addition, cortisol results were compared to stressed (45-65 ng mL^{-1}) and non-stressed (10-25 ng mL^{-1}) plasma cortisol concentrations in a closely related acanthurid (*Ctenochaetus striatus*) (Soares et al., 2011).

Statistical Methods

Statistical analyses were performed using 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 two-way ANOVA was used, with decompression treatment and venting as fixed factors and replicate block as a random factor, to compare mean cortisol concentrations. Tukey's multiple comparisons test was used to determine significant differences between levels within each factor.

RESULTS

Fish Sizes

The size of *Z. flavescens* used in the study ranged from 5.0-10.0 cm standard length (SL) with a mean value of 7.2 ± 0.9 cm (Appendix Table 3, Appendix Figure 1), similar to sizes previously reported in the West Hawaii fishery (Stevenson, et al., 2011)

Mortality

Mortality occurred <24 h post-collection only in fish subjected to fast decompression with no venting, with a mean mortality of 6.2% (SE, 0.6%, Figure 1). No mortality occurred in the other experimental treatments, negating the need for statistical tests.

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, exophthalmia, and protrusion of the intestines from the cloaca in fish subjected to fast decompression (Figure 2). The intermediate decompression treatment resulted in some fish being bloated and positively buoyant, but the other symptoms were not as frequently observed. Fish subjected to slow decompression did not exhibit these signs.

Histopathology

Histopathology of the 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 either pre- or post-collection. However, in one case a venting wound was detected in a fish subjected to slow decompression and venting, which was sampled immediately after collection. In this sample (Figure 3), there was locally extensive necrosis of body wall musculature and a localized influx of neutrophils surrounding the needle track.

Cortisol

The mean ocean baseline cortisol concentration was 8.9 ng mL^{-1}, *SE*= 4.96 ng mL^{-1} and in most some cases was at or below the 3.9 ng mL^{-1} detection limit for the assay. Decompression treatment significantly affected cortisol concentration (Two-way ANOVA: F=4.26; df= 2,10; p=0.03), with fast decompression resulting in a significantly higher mean cortisol concentration (M=58.8 ng mL^{-1}, SE=8.7 ng mL^{-1}) than slow decompression (M=35.5 ng mL^{-1}, SE=5.3 ng mL^{-1}), with neither treatment being significantly different from intermediate decompression (Figure 4). Fast decompression produced the highest observed cortisol concentration (101.49 ng mL^{-1}), whereas the highest observed cortisol concentrations in fish subjected to intermediate and slow decompression were 59.09 and 68.03 ng mL^{-1}, respectively. Venting resulted in a slightly 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}), but differences were not significant (Two-way ANOVA: F=0.90; df=1,20; p=0.36). There was no significant interaction between decompression and venting (Two-way ANOVA: F=1.54; df=2,20; p=0.239).

Comparisons between post-collection cortisol concentrations in *Z. flavescens* and the ocean baseline concentration, and those in *C. striatus* suggest that all treatments produced

elevated cortisol above 'non-stressed' levels. However, only the fast decompression and venting treatment resulted in a mean cortisol concentration in the 'stressed' range.

DISCUSSION

The conflict between fishers and opponents of aquarium collecting in Hawaii is not only driven by the competition for tropical fish, but also by values differences (Capitini et al., 2004; Tissot, 2005; Stevenson et al., in review). Because values are involved, science alone may not be sufficient to settle the dispute. However, because conflict surrounding the aquarium trade also suffers from scientific uncertainty (Capitini et al., 2004), we aim to provide the science necessary for full evaluation of potential management solutions directed at collection practices.

With the objective of informing management on collection practices in the West Hawaii aquarium trade, our study focused on the short- and long-term mortality of reef fish subjected to two different barotrauma mitigation practices: venting and decompression. Overall, we found that varying decompression methods produced little to no mortality and that venting reduced mortality in fish subjected to fast decompression. Furthermore, we found no evidence of significant tissue inflammation associated with venting, nor lesions linked to barotrauma immediately after collection and after a 21 d holding period. Finally, decompression significantly elevated serum cortisol concentration above baseline values, with fast decompression resulting in significantly higher serum cortisol concentrations than slow decompression. However, consistent with mortality observations, venting did not significantly affect cortisol concentration. In the following sections, we discuss possible factors influencing our results, future research recommendations, and implications for management of aquarium fisheries.

Mortality

Only fish subjected to fast decompression with no venting suffered post-collection mortality, while a popular method used to collect yellow tangs in the West Hawaii fishery, fast decompression followed by venting, resulted in no mortality. Our findings are consistent with the results of Stevenson et al. (2011), who reported low mortality rates in *Z. flavescens* in the West Hawaii fishery. Our results indicate that venting following decompression does not cause short- (0 d post-collection) or longer-term (21 d post-collection) mortality. Venting likely improved survivorship in fish following fast decompression because venting causes fish to become neutrally or negatively buoyant, allowing them to control position in the water and avoid colliding with the transport container during transport to the holding facility. This is contrasted with fish subjected to fast decompression with no venting, which were positively buoyant and therefore at risk of acquiring secondary injuries during transport, as well as aerial exposure.

Additional factors that influence post-collection fish mortality include collection depth, body size, and species. Our study examined fish collected from 15-18m depths, which is typical for most West Hawaii fishers, although some dive to ≥27m for different species (Stevenson et al., 2011). At these deeper depths, the effects of decompression rate and venting may be quite different, and fish mortality and occurrence of barotrauma increases with capture depth (Collins, et al., 1999; St John & Seyers, 2005; Hannah, et al., 2008; Jarvis & Lowe, 2008; Campbell, et al., 2010). Interviews with West Hawaii fishers indicate that fish collected from >25m require more decompression time and venting 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 deep water fish (Hannah et al., 2008; St John & Seyers, 2005). Also similar to deep water fish that exhibit different responses to decompression (Hannah and Matteson, 2007; Jarvis and Lowe, 2008;

Pribyl, 2010), aquarium fish species react differently to decompression and venting. These differences are likely caused by variation in body shape and durability, and swim bladder volume between species. Methods of fishers reflect these species differences, and they implement different collection methods for different species, 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 showed some localized inflammation, with no visible bacteria. Because the microtome used in the histological process sliced tissue into a 5um sections, there may have been other fish with similar minor wounds that were undetected. However, the object of histopathology in our study was to determine if widespread inflammation or tissue damage were present in fish, indicating serious sublethal injury. If such injuries were present, they would have been detected in multiple sections of the tissues surrounding the venting wound. The fish in which the venting wound was detected was sampled 0 d post-collection, and serious infection is not be expected within hours of collection. Rather, inflammatory and epithelial cells mainly occupy the area of the wound within a few hours of injury (Roberts, 2010), and invading bacteria would not have spread far from the wound in this time.

If fish were unable to heal in the 21 d holding period because of wound severity, we would have detected significant infection in fish sampled after 21 d. Because we did not, our results indicate that the venting procedure does not pose a significant threat to fish survival post-collection, nor cause significant sublethal effects. However, we caution that the fish in our study were held in an aquaculture facility for 21 d without additional handling and transport stressors as they normally would in the supply chain, thus potentially promoting recovery from injuries inflicted during collection. Future studies should investigate if 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 deep-water temperate fish, we expected internal barotrauma symptoms to be present similar to those described by Pribyl (2010). We did not detect tissue-level signs of barotrauma, even in fish subjected to fast decompression. However, externally observable signs indicated that barotrauma did occur and that aquarium fish likely also experience some internal signs of barotrauma. Because positively buoyant fish were bloated and showed protrusion of the intestines from the cloaca, with some presenting exophthalmia, organ displacement by the swim bladder and stretching of the optic nerve caused by exophthalmia were likely occurring immediately following collection, internal barotrauma signs detected in rockfish (Parker et al., 2006; 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 fast decompression.

Cortisol

Fast decompression produced significantly higher cortisol levels in fish than slow decompression. This higher stress level may be driven by a higher frequency and severity of

barotrauma symptoms in fish subjected to fast decompression. Because fish endured transport to the holding facility prior to blood collection, fish exhibiting positive buoyancy (those not subjected to venting) should have produced higher cortisol concentrations because they were more susceptible to injury and stress from collision with the transport container. Venting, applied prior to transport, should reduce exposure to this transport stressor by eliminating positive buoyancy. However, our results show that venting by itself did not significantly affect cortisol concentration. This suggests that other signs of barotrauma not relieved by venting (i.e. intestinal protrusion and exophthalmia), were likely drivers of higher stress levels in fish subjected to fast decompression. Comparisons with Soares et al. (2011) "non-stressed" cortisol levels in *Ctenochaetus striatus* suggest that venting increases stress in fish subjected to fast decompression, although it also reduces mortality. Because fast decompression followed by venting is a popular method used to collect yellow tangs in West Hawaii, further investigation of stress following collection is needed.

Handling in and transport between export, import, and retail facilities may exacerbate stress caused by collection. Chronic stress results in immune system suppression (Barton, 2002), increasing susceptibility to infection and disease and the probability of delayed mortality. Because mortality occurring in hobbyist aquariums followed by fish replacement is one driver of the demand for more aquarium fish (Tissot et al., 2010), future studies should address stress as it relates to handling in and transport between each link in the supply chain beyond collection. In addition, tracking supply chains originating both inside and outside the US and investigating how fish stress relates to water quality, fish behavior, sublethal effects, and mortality would provide insight into drivers of stress and mortality in the aquarium fish trade and lead to improved methods.

Implications for Management

Venting did not cause mortality or sublethal injuries in yellow tangs, and banning venting may increase mortality rates if fishers continued to implement fast decompression. In addition to increasing fish mortality, banning venting and only allowing slow decompression might be economically detrimental to fishers. Our results further our overall understanding of the effects of venting. Previous studies show conflicting results on venting's effectiveness, both supporting and refuting the efficacy of its use for reducing 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, although our inference is limited to a single species.

In contrast to other aquarium fisheries such as in the Philippines where cyanide is used and increases fish and coral reef mortality (Hall & Bellwood, 1995; Hanawa et al., 1998; Rubec et al., 2001; Jones & Hoegh-Guldberg, 1999; Jones & Steven, 1997), fishers in West Hawaii appear to implement non-destructive collection practices. Although the Hawaii fishery contributes fewer ornamental aquarium fish to the global exported catch relative to Indonesia and the Philippines (Wood, 2001; Walsh, et al. 2004), if methods used by fishers in West Hawaii were adopted by these large scale aquarium fisheries where destructive fishing methods are popular, sustainability of the global trade would improve. To this end, we recommend that other aquarium fisheries adopt methods used in West Hawaii, which are economically feasible and foster sustainability.

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FIGURES

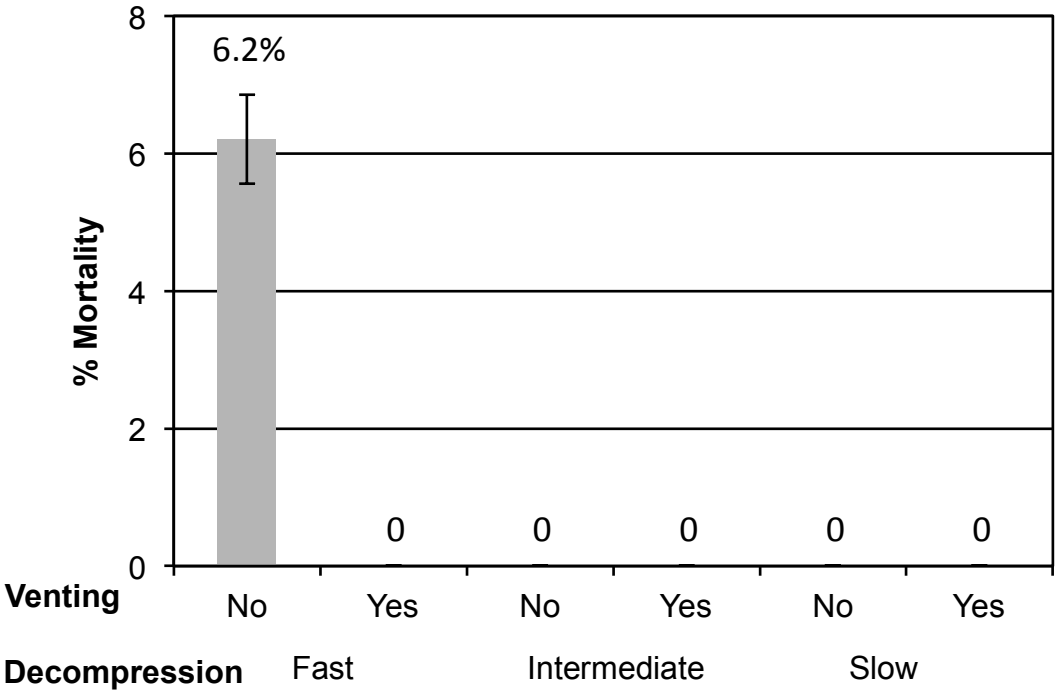

Figure 1: Percentage of fish mortality by treatment group in yellow tangs collected in West Hawaii.

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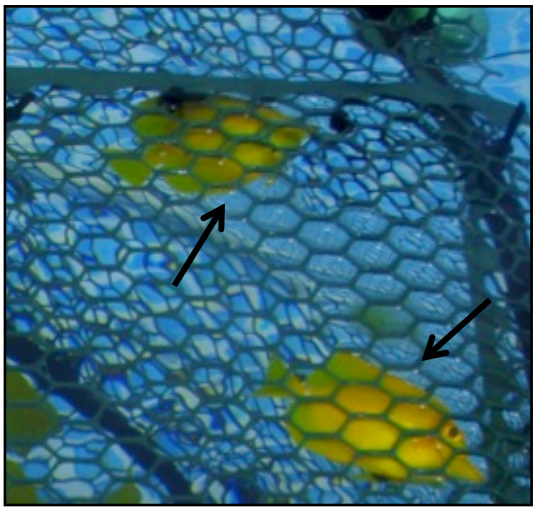

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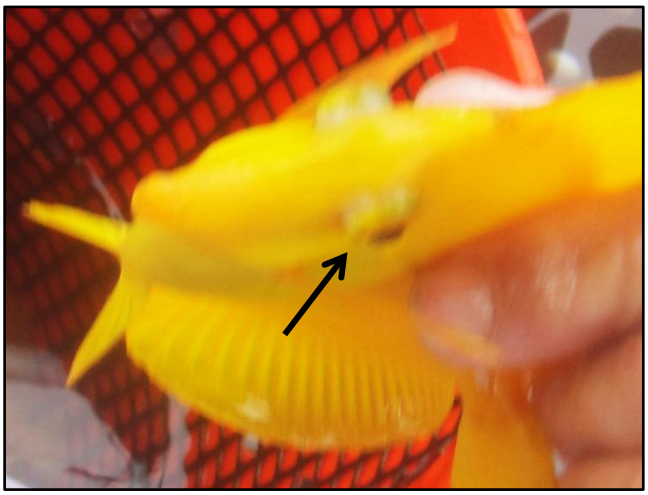

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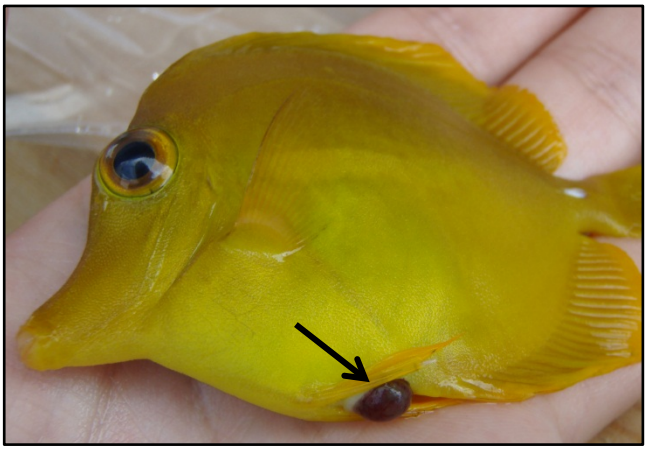


Figure 2: Barotrauma signs observed in yellow tangs following collection: (a) positive buoyancy (b) exophthalmia and (c) intestinal protrusion from the cloaca.

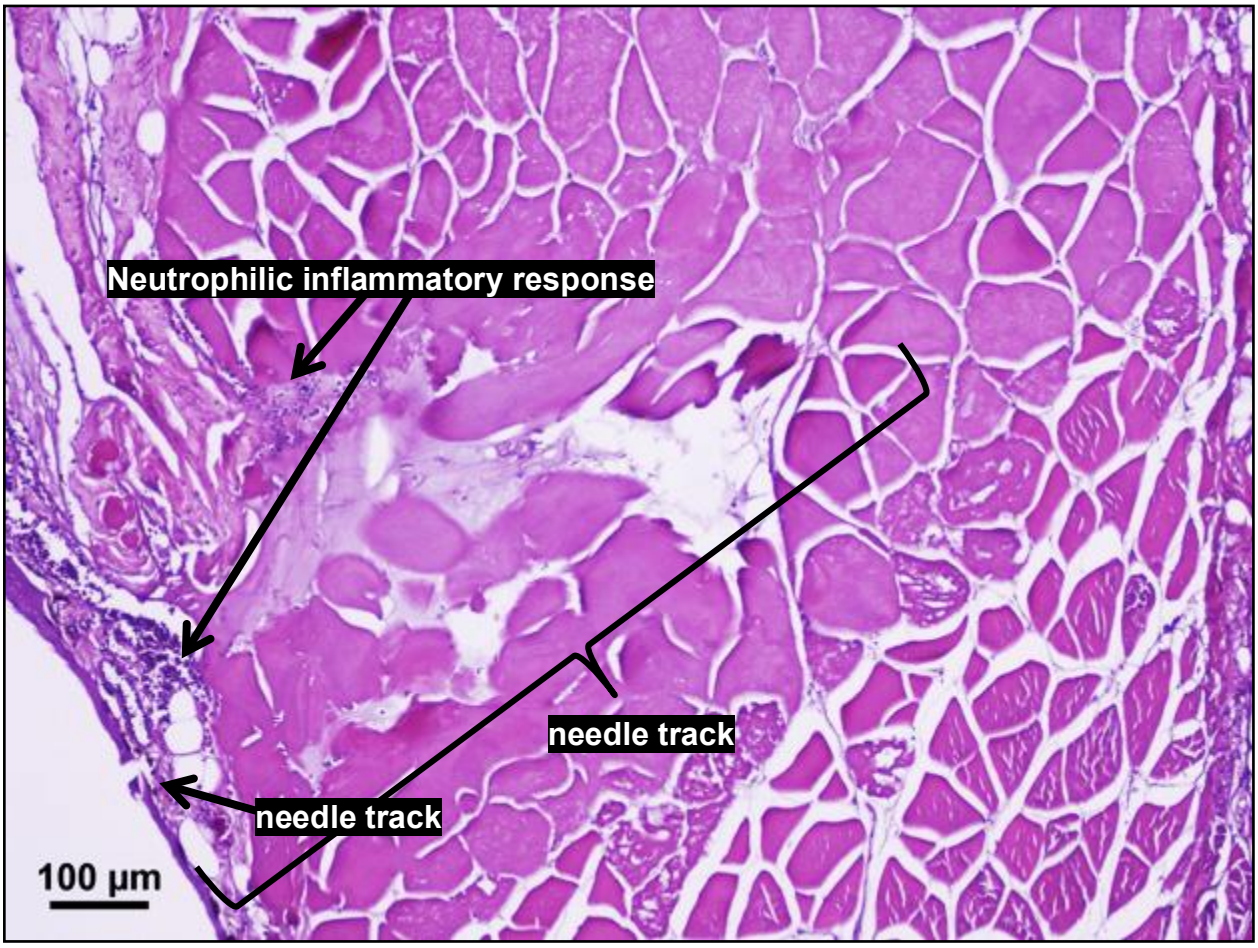


Figure 3: Histological section of needle track in a yellow tang subjected to venting showing muscle cell necrosis, edema, and neutrophilic inflammation, at 10x magnification.

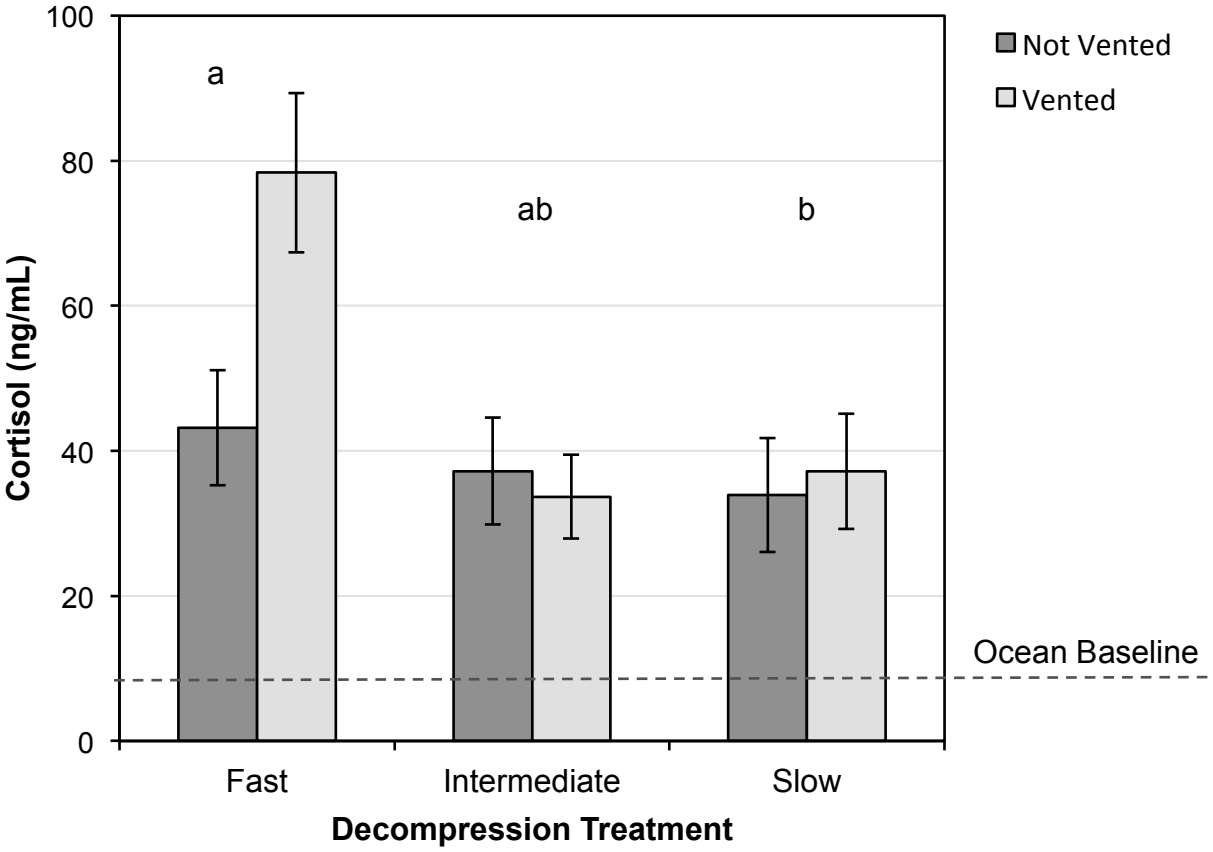


Figure 4: Cortisol concentration (mean +/-SE) by each treatment. Letter groups represent Tukey's multiple range test results comparing means between decompression treatments. All treatment groups are above the ocean baseline concentration of 8.9 ng mL^{-1}.

APPENDIX

TABLES

Table 1: Descriptions of the six experimental treatments.

		Venting Treatment	
		Yes	**No**
Decompression Treatment	**Fast**	Fish brought directly to surface from depth Duration: ~1 minute Vented	Fish brought directly to surface from depth Duration: ~1 minute Not Vented
	Intermediate	Fish brought from depth to 6 meters and allowed to decompress for 45 minutes Duration: 45 minutes Vented	Fish brought from depth to 6 meters and allowed to decompress for 45 minutes Duration: 45 minutes Not Vented
	Slow	Fish brought up from depth in increments of 3m until 10 m reached, then in increments of 1.5 m and allowed to decompress for 15 minutes at each stop Duration: >2 hours Vented	Fish brought up from depth in increments of 3m until 10 m reached, then in increments of 1.5 m and allowed to decompress for 15 minutes at each stop Duration: >2 hours Not Vented

Table 2: Water quality measurements and animal husbandry log for each pool over the course of the 21 day holding period. Most measurements were conducted twice each day, in the morning and afternoon hours in order to determine the approximate daily maximum and minimum water temperature. Numbers 1, 2, and 3 indicate each replicate block pool. Grayed areas indicate that the experiment in that pool had not begun, or had been completed.

Date	Time	Filter Change	Pool Cleaned			Temperature (°C)			Salinity			Food			Comments		
			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
7/11/11	10:00	x				27.5	27.5		35	35		*Ulva* in cage	*Ulva* in cage		Start experiment for all fish in Pool 1. Not eating.	Start experiment for ID & FD fish in Pool 2. Not eating	
7/11/11	16:38					32.0	31.2		35	35		"	"		ID – V & SD + V eating	ID – V & SD + V eating	
7/12/11	10:00					27.5	28.0		35	35		"	"		Ate almost all food	All ate most food	
7/12/11	16:32		x			31.5	30.0		35	35		"	"		Ate all food	Ate all food except SD – V.	
7/13/11	8:30	x				27.0	27.5		35	35		"	"		Eating a lot	Eating a lot	
7/13/11	18:00					31.5	32.0		35	35		"	"		Eating a lot	Eating a lot	
7/14/11	10:00					27.5	28.0		35	35		"	"		Eating a lot	Eating a lot	
7/14/11	18:00					29.0	29.5		35	35		"	"		Ate all food	Ate all food	
7/15/11	10:00	x				27.5	28.0		35	35		"	"				
7/15/11	18:00		x	x		31.5	31.5		35	35		"	"				
7/16/11	9:00					28.5	28.5		35	35		"	"				
7/16/11	17:00					32.0	31.0		35	35		"	"				
7/17/11	-																
7/17/11	16:00					31.3	32		35	35		"	"			Start experiment for FD fish in Pool 2.	Start experiment for all fish in Pool 3.
7/18/11	9:00	x				28	28.0	28.5	35	35	35	"	"	*Ulva* in cage	All eating	FD fish not eating	Not eating
7/18/11	17:00					29.5	29.5	29.5	35	35	35	"	"	Same f/ morning		FD - V not eating; FD + V ate some	Not eating
7/19/11	10:00		x			28.5	28.0	28	35	35	35	"	"	*Ulva* in cage		Eating some (new fish)	Eating some
7/19/11	18:00					31	31.5	30.5	35	35	35	"	"	Same f/ morning	All eating	FD fish not eating much	Only SD fish eating
7/20/11	7:40	x		x		28	28.0	28	35	35	35	"	"	Same f/ 7/19	SD fish didn't eat all food	FD fish not eating much.	Not eating much

7/20/11	19:30								35	35	35	"	"		All eating, look healthy	All eating	All eating
7/21/11	10:30					31	31.5	31	35	35	35	"	"	*Ulva* in cage	All eating, look healthy	All eating, look healthy	
7/21/11	19:30								35	35	35	"	"	"			
7/22/11	11:00	x				31.5	31.5	31.5	35	35	35	"	"	"			SD + V & ID + V didn't eat all food
7/22/11	-								35	35	35						
7/23/11	10:00				x	30	30	30.5	35	35	35	"	"	"		FD – V didn't eat all food	FD – V didn't eat all food
7/23/11	17:00					31.5	31	32.5	35	35	35	"	"	"	Ate all food	Ate all food	Ate all food
7/24/11	12:00					31.5	32	32	35	35	35	"	"	"			
7/24/11	15:00					33	33	33	33	35	35	"	"	"	All eating		
7/25/11	11:00	x				31.5	31.5	31.5	30	35	35	*Ulva* top of cage	*Ulva* top of cage	*Ulva* top of cage			
7/25/11	16:00					33.5	33.5	33.5	31	35	35	"	"	"			
7/26/11	7:30					29.5	30	30	31	35	35	"	"	"			
7/26/11	17:00					33	33.5	33.5	33	35	35	"	"	"			
7/27/11	11:00	x				32	32	32.5	34	35	35	"	"	"			
7/27/11	16:00					32	32.5	32.5	34	35	35	"	"	"			
7/28/11	7:30					29.5	29.5	29.5	34	35	35	"	"	"			
7/28/11	16:00					32.5	33	33.5	35	35	35	"	"	"			
7/29/11	12:30	x			x	32	32	32	35	35	35	Nori top of cage	Nori top of cage	Nori top of cage			
7/29/11	16:00								35	35	35	"	"	"			
7/30/11	11:30					32.5	32.5	32.5	35	35	35	"	"	"			
7/30/11	15:00					33.5	33.5	33.5	35	35	35	"	"	"			
7/31/11	10:30					32.5	32	32	35	35	35	"	"	"			

7/31/11	15:00		x			33	33	33	34	35	35	"	"	"			
8/1/11	10:40	x				29	29	29		35	35		"	"	End experiment Pool 1.	End experiment for ID & SD in Pool 2.	
8/1/11	19:00							27		35	35		"	"			
8/2/11	11:00	fw				28.5	29	28.5		35	35		"	"		Start hyposalinity treatment.	
8/2/11	16:00									34	35		"	"			
8/3/11	7:30	x				26	26	26		27	35		"	"			
8/3/11	16:00									27	35		"	"			Eating a lot
8/4/11	8:00									23	35		"	"			
8/4/11	17:00					31	31	30		23	35		"	"			
8/5/11	9:30	x				28	28	28		16	35		"	"			Eating a lot, Look healthy
8/5/11	17:00					31.5	32	32		14	35		"	"			Start hyposalinity treatment. Eating a lot.
8/6/11	9:00					27.5	28	28		15	28		"	"			Eating a lot, look healthy
8/6/11	18:30					31.5	31.5	31		16	23		"	"			
8/7/11	10:00									20	21		Nori top of cage f/ 8/6	"			All eating, reactive
8/7/11	14:30					32.5	32	32		20	16		Nori top of cage for FD fish	"			
8/8/11	10:00						32	32		14	14					End experiment for FD in Pool 2.	End experiment for all fish in Pool 3.

Table 3: Mean fish standard length for each replicate group within each treatment. Values are mean ± the sample standard deviation. The minimum and maximum standard lengths for each group are also shown.

Table 5: Fish standard lengths						
Decompression treatment	**Venting (Y/N)**	**Replicate**	**n**	**Mean length SL ± SD (cm)**	**Minimum SL (cm)**	**Maximum SL (cm)**
None	N	1	26	6.7 ± 0.8	5.3	8.4
		2	21	7.8 ± 1.2	5.3	9.8
		3	22	7.2 ± 1.0	6.0	9.1
	Y	1	25	6.9 ± 0.7	5.5	8.0
		2	21	8.1 ± 0.9	5.6	9.9
		3	22	7.6 ± 0.8	6.3	8.8
Intermediate	N	1	22	6.6 ± 0.7	5.6	7.9
		2	21	6.8 ± 0.8	5.3	8.3
		3	20	7.5 ± 0.7	6.5	9.3
	Y	1	22	7.0 ± 0.8	5.9	8.3
		2	25	6.9 ± 0.8	5.6	8.9
		3	22	7.6 ± 0.9	6.0	8.9
Slow	N	1	20	6.8 ± 0.5	6.0	7.6
		2	19	6.7 ± 0.8	5.6	8.5
		3	24	7.8 ± 1.1	5.3	10.0
	Y	1	20	6.9 ± 0.7	5.6	7.9
		2	22	6.8 ± 0.6	5.5	7.5
		3	24	7.8 ± 0.7	5.9	8.8
Overall			398	7.2 ± 0.9	5.3	10.0

Table 4: Review of venting literature.

Table 1: Studies examining venting as an effective barotrauma mitigation measure and their results.

Species	Sample Size (n)	Depth (m)	Hypodermic Needle Gauge	Observation Period and Method	Venting Benefit	Venting Detriment	Conclusion: Is venting beneficial?	Comments	Source
Blue Rockfish (*Sebastes mystinus*)	4884 total all depths	0-91	18	0- 730 day recapture	Greater tagged returns of vented fish from 46-61m depth range	Fewer tagged returns of vented fish from 0-46m and 61-91m ranges	Inconclusive	Aquarium-held vented fish had high long-term survival (2 years). Mentions venting as a valuable tool for aquarium fish collection.	Gotshall (1964)
Burbot (*Lota lota*)	103 over 9 experi-ment days	>10	NA	4 days survival at depth in cages; 0-150 day recapture	Equal survival and recapture of vented and non-vented fish		Inconclusive	Effects of barotrauma are lessened if fish are quickly recompressed. Percentage of burbot requiring a second venting treatment after recapture increased with time spent at large, indicating that the swimbladder healed over a period of 27 days.	Bruesewitz et al. (1993)
Yellow Perch (*Perca flavescens*)	169 over 5 experi-ment days	10-15	NA	8 days in holding facility, but only first 3 days used in analysis; 33-597 day recapture	Venting increased survival during short-term	Vented fish had lower recapture rates over long-term	Inconclusive	Decompression treatment had higher survival than non-decompressed fish, though this differed between the two depths.	Keniry et al. (1996)
Largemouth Bass (*Micropterus salmoides*)	10	5, 8.5, 11	18	28 days	No venting mortality, swim bladder resumes function following venting		It is not harmful.	Fish subjected to 0.5cm puncture wounds were negatively buoyant, but vented fish were not. Recommend venting for this species if caught >6 m depth	Shasteen & Sheehan (1997)
Smallmouth bass (*Micropterus dolomieu*)	9	< or = 49	21	4 days constant tracking with telemetry	Venting did not result in mortality	Not venting did not result in mortality	Inconclusive	Venting helped fish resubmerge, but made them negatively buoyant.	Nguyen et al. (2009)
Many (review)	39 studies	NA	NA		Increased survival for 5 of 39 examined species	Decreased survival for 2 of 39 examined species	Inconclusive	Relative risk of mortality (survival of treatment/survival control) lower at shallower depths. No distinction between long-term versus short-term studies examining survival. No difference between ability of anglers versus fisheries biologists in venting success (measured as risk).	Wilde (2009)

FIGURES

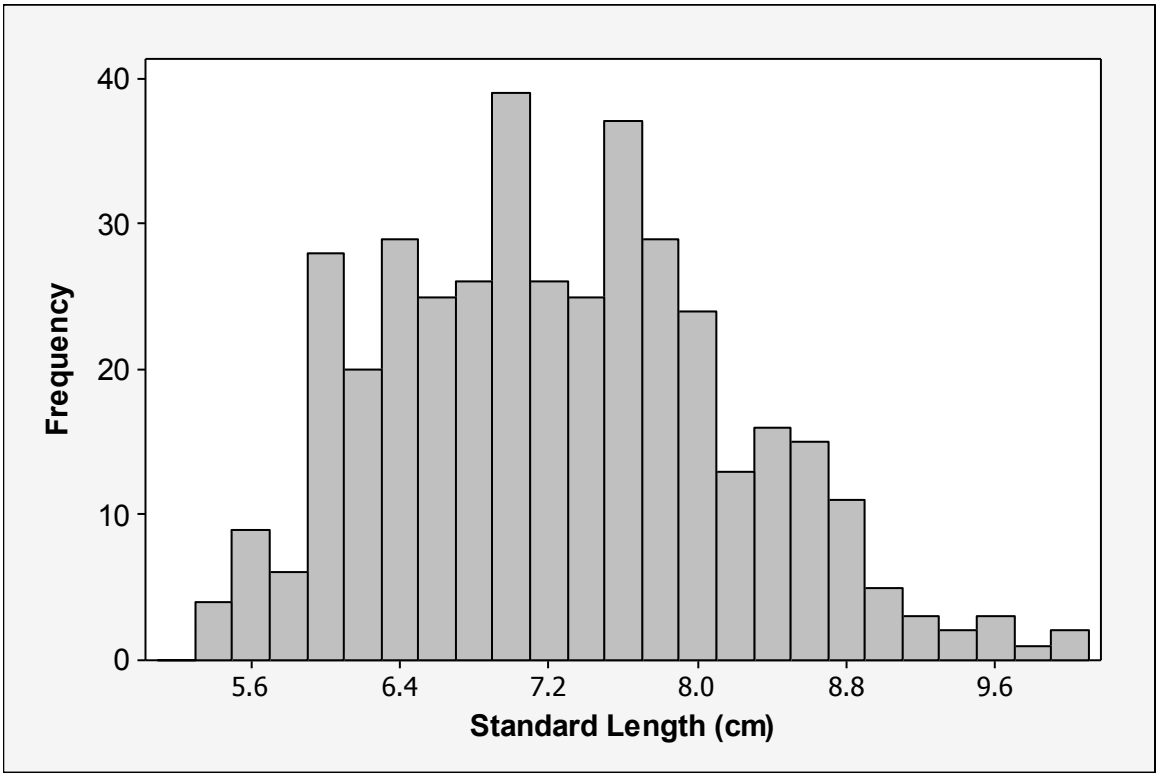


Figure 1: Standard length frequencies of all yellow tangs used in experiment.

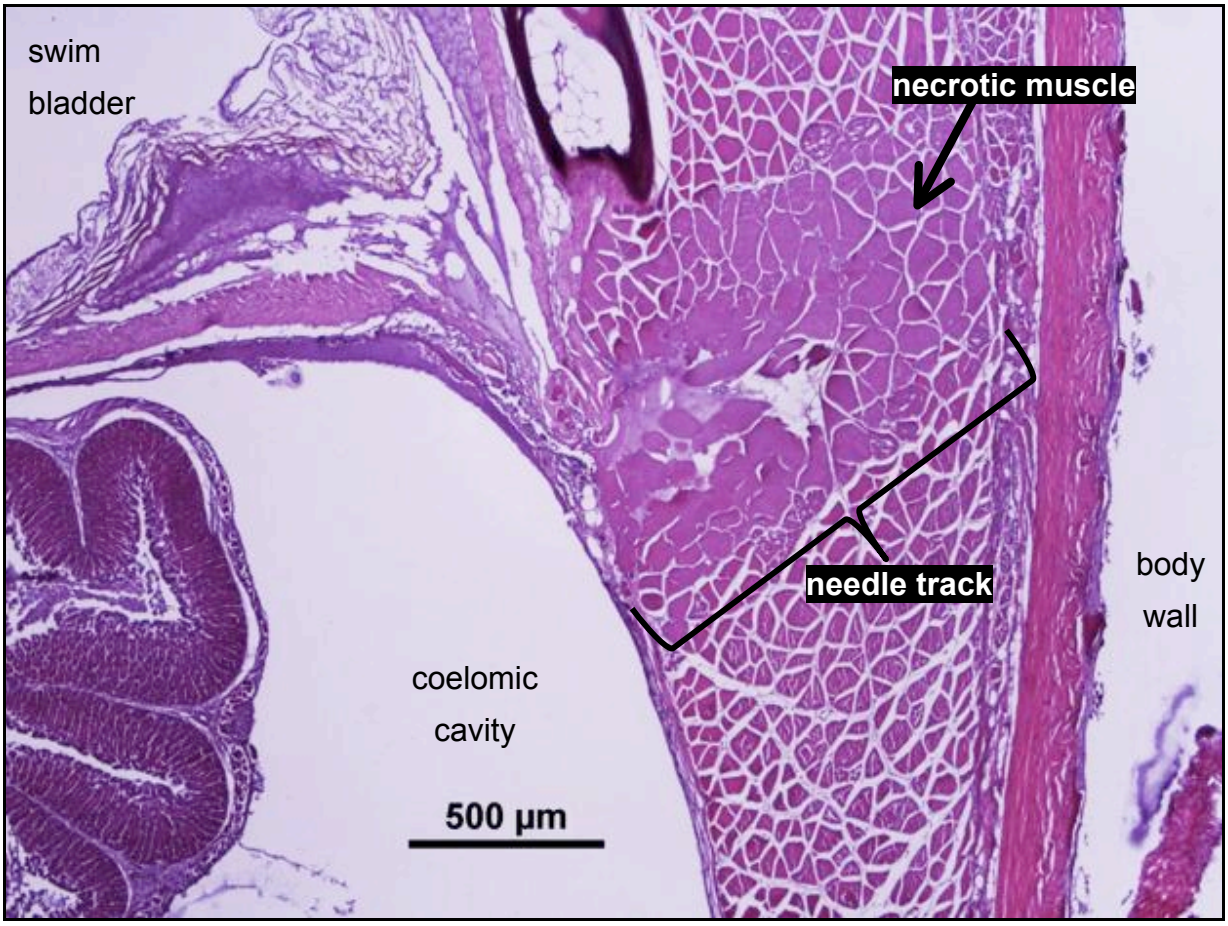
a.

Figure 2: Venting performed on yellow tangs by experienced fisher.

Figure 3: Comparison of buoyancy in yellow tangs subjected to the fast decompression treatment, before and after venting was applied by an experienced aquarium fisher.

Figure 4: Holding facility at the National Energy Laboratory Hawaii Authority (NELHA

a.


swim
bladder
necrotic muscle
needle track
body
wall
coelomic
cavity
500 µm

b.

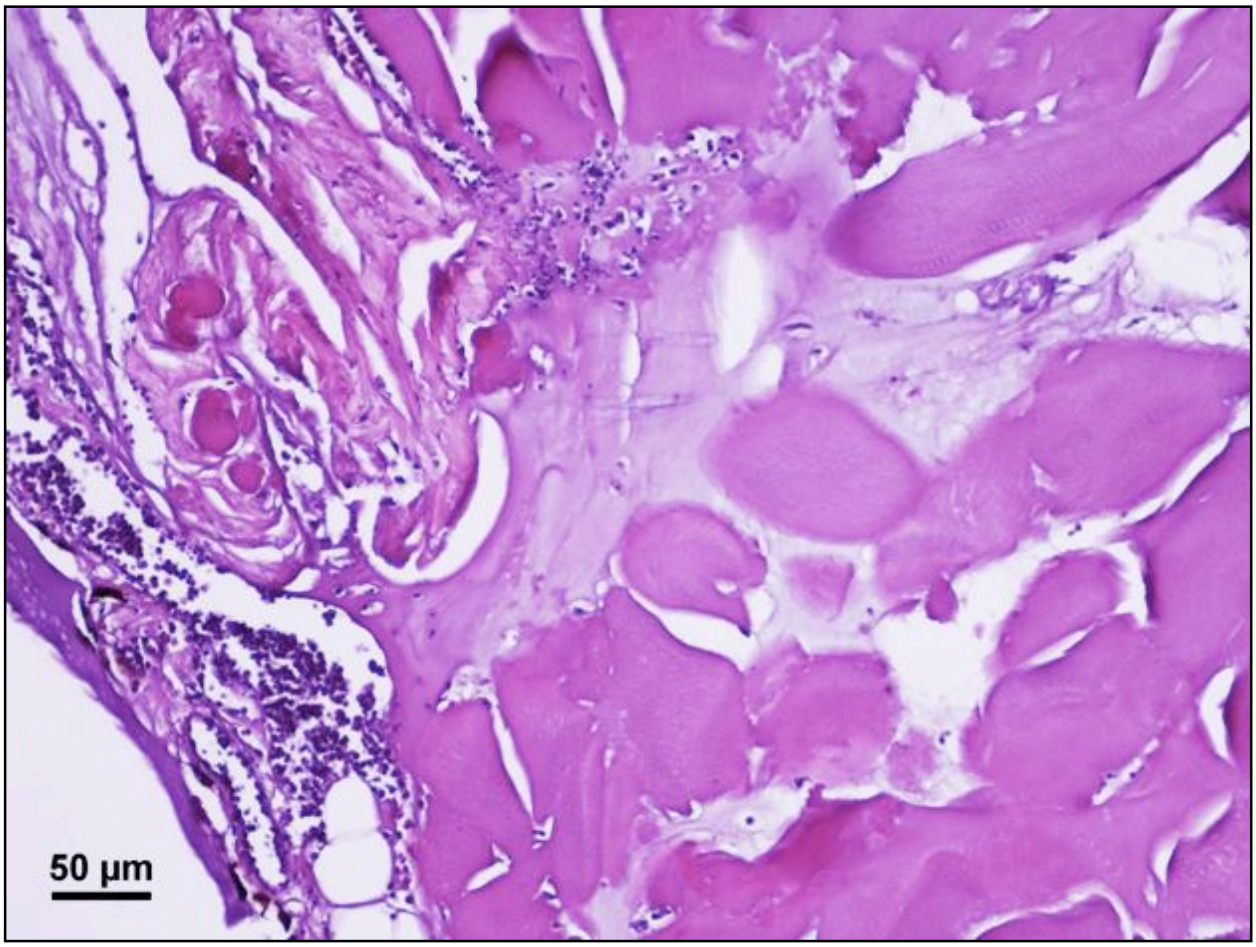


Figure 5: Histological section of a needle track in a yellow tang showing surrounding necrotic tissue and neutrophilic inflammatory response, at (a) 4x and (b) 40x magnification.