

A peer-reviewed version of this preprint was published in PeerJ on 17 February 2015.

[View the peer-reviewed version](https://peerj.com/articles/756) (peerj.com/articles/756), which is the preferred citable publication unless you specifically need to cite this preprint.

Munday ES, Tissot BN, Heidel JR, Miller-Morgan T. 2015. The effects of venting and decompression on Yellow Tang (*Zebrasoma flavescens*) in the marine ornamental aquarium fish trade. PeerJ 3:e756
<https://doi.org/10.7717/peerj.756>

The effects of venting and decompression on Yellow Tang (*Zebrasoma flavescens*) in the ornamental aquarium fish trade

Each year, over 45 countries export 30 million fish from coral reefs as part of the global marine ornamental aquarium trade. This catch volume is affected by collection methods that cause mortality. Barotrauma caused by forced ascent of collected fish from depth has been determined to be a cause of post-collection mortality. The detrimental effects of barotrauma can be prevented by decompression, or mitigated with venting (puncturing the swim bladder to release expanded internal gas). To further evaluate the effects of collection methods on fish stress and mortality, we conducted the first comprehensive study on the effects of barotrauma prevention and mitigation practices on marine ornamental fish. We examined the effects of three ascent treatments, each with decompression stops of different frequency and duration coupled with or without venting, on sublethal effects and mortality in yellow tang (*Zebrasoma flavescens*), using histology and serum cortisol measurements. In *Z. flavescens* subjected to ascent without decompression stops or venting, a mean post-collection mortality of 6.2% occurred within 24h of capture. Common collection methods in the fishery, ascent without or with one decompression stop followed by venting, resulted in no mortality. Histopathologic examination of heart, liver, head kidney, and swim bladder tissues in fish 0d and 21d post-collection revealed no significant lesions in any treatment group. Ascent without decompression stops resulted in significantly higher serum cortisol than ascent with many decompression stops, and venting alone did not affect cortisol. Future work should examine links in the supply chain following collection to determine if further handling and transport stressors affect survivorship and sublethal effects.

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

3 Emily S. Munday^{1*}, Brian N. Tissot², Jerry R. Heidel³, and Tim Miller-Morgan^{3,4}

4 *Corresponding author: Montana Tech of the University of Montana, 1300 W Park Street, Butte,
5 MT, 59701, USA. Email: emily.munday@gmail.com, Tel: 1 + 857-919-1899 Fax: 406-496-4696

6 ¹School of the Environment, Washington State University Vancouver, Vancouver, WA, 98686
7 and Montana Tech of the University of Montana, 1300 W Park Street, Butte, MT, 59701

8 ²Humboldt State University Marine Laboratory, 570 Ewing St., Trinidad, CA 95570

9 ³College of Veterinary Medicine, Oregon State University, 700 SW 30th Street, Corvallis, OR,
10 97331, USA

11 ⁴ Aquatic Animal Health Program, Oregon Sea Grant, Hatfield Marine Science Center, 2030 SE
12 Marine Science Drive, Newport, OR 97365

13
14
15
16
17
18
19
20
21
22

23 Introduction

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

34 Aquarium fisheries that utilize destructive fishing practices have high mortality. In the
35 Philippines and Indonesia where cyanide is used to stun ornamental fish for ease of capture, >90
36 % of fish suffer mortality and coral reefs are severely damaged (Hall and Bellwood 1995;
37 Hanawa et al. 1998; Rubec et al. 2001; Rubec and Cruz 2005; Jones and Hoegh-Guldberg 1999;
38 Jones and Steven, 1997). While fishers in Hawaii do not use cyanide to collect fish, and
39 immediate mortality is low (<1%) (Stevenson et al. 2011), it is possible that fishers' collection
40 methods result in delayed mortality. Because fish move rapidly through the supply chain, it is
41 possible that aquarium fishers are unaware of collection methods that result in mortality further
42 along the supply chain. Economically, delayed mortality shifts the burden of fish death and
43 monetary loss from the collector to those further along the supply chain (e.g. the importer, or
44 hobbyist) while also increasing the demand for fish and exacerbating pressure on coral reef
45 ecosystems. Identifying methods that cause delayed mortality would reduce the overall mortality

46 of aquarium fish in the aquarium trade, and thus the number of fish removed from the reef to
47 compensate for these losses.

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

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

64 Research on deeper dwelling food fishes has revealed that protrusion of the esophagus from the
65 mouth is common (Parker et al. 2006; Pribyl 2010; Wilde 2009). In addition, internal signs of
66 barotrauma such as swim bladder rupture, internal bleeding, compression of and damage to
67 organs surrounding the swim bladder, stretching of optic nerves, emphysema of the heart

68 ventricle and epithelial surfaces, and gas emboli in the rete mirabile and kidney caused by gas
69 leakage from the swim bladder (Gotshall 1964; Bruesewitz et al. 1993; Parker et al. 2006;
70 Rogers et al. 2008; Pribyl 2010).

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

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

91 evaluate the efficacy of each of these procedures in preventing mortality. While the effects of
92 venting and decompression on aquarium fish has not been documented, these methods have been
93 fairly well studied in deeper-dwelling food fishes.

94 In these deeper-dwelling fishes, decompression takes a long time – up to several days – which is
95 a direct result of the depths these fish are removed from (Parker et al. 2006; Pribyl 2010).

96 Decompression is a time-consuming process because in order to prevent barotrauma, one must
97 allow adequate time for fish to naturally remove the expanding swim bladder. Likewise, in
98 Hawaii, decompression can be prohibitively time-consuming for fishers to implement; even for
99 shallow-dwelling reef fish. The time-consuming nature of decompression deters fishers who
100 would rather remove fish quickly from depth so as to return to depth and collect more fish.
101 However, bringing fish up to the surface quickly without decompression stops results in
102 barotrauma. To mitigate barotrauma, fishers use venting.

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

111 As previously stated, fishers often use some combination of decompression and venting. For
112 example, it is common practice for aquarium fishers to perform one or several decompression

113 stops, pausing in the water column at intermediate depths before removal to the surface (LeGore
114 et al. 2005; Stevenson et al. 2011). In Hawaii, fishers typically vent the fish following this
115 practice.

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

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

129 **Methods**

130 **Experimental Design**

131 The Yellow Tang (*Z. flavescens*) was selected as the study animal because it is the most
132 commonly targeted aquarium species in West Hawaii, consistently composing nearly 80% of the
133 total catch of aquarium fish there (Cesar et al. 2002; Tissot and Hallacher 2003; Walsh et al.
134 2004; Williams et al. 2009). Therefore, understanding how collection practices affect Yellow

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

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

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

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

166 As is typical in the fishery, venting was performed by the fisher on the fishing vessel using a 20
167 G hypodermic needle, replaced after approximately 50 fish. Each fish was held out of water for
168 $<3 \text{ s}$ by the fisher while the needle was inserted through the body wall toward the swim bladder,
169 caudal to the pectoral fin and ventral of the lateral line.

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

173 **Holding Period**

174 Post-collection, fish were observed for 21 d at an aquaculture facility located at the Natural
175 Energy Laboratory Hawaii Authority in West Hawaii provided with natural surface seawater at
176 ambient temperatures. The experiment duration was chosen because after interviewing West
177 Hawaii fishers, it was determined that 21d represents a reasonable time period for a fish to be

178 transferred from the reef to a retailer or hobbyist in this particular supply chain. In addition,
179 swim bladder healing in rockfish has been observed after 21 d (Parker et al. 2006) and is
180 sufficient time to allow skin and muscle regeneration in fish (Roberts 2010). Therefore, fish
181 exhibiting lesions after 21 d may not have fully recovered in a supply chain environment and
182 could be categorized as having sublethal effects from collection.

183 Fish were held in 1 m diameter mesh floating cages within three 10,000 l pools, which served as
184 replicate blocks, each containing all six treatments. Incoming seawater was filtered to 5 μ m, and
185 set to flow through each pool at a rate of 1 volume \square d⁻¹. Pools were exposed to natural sunlight,
186 and temperatures was measured twice daily.

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

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

197 **Histopathology**

198 To determine the sublethal effects of collection methods, fish (n=5) were chosen randomly from
199 each replicate treatment group immediately upon arrival to the holding facility (0 d) and at the

200 end of the holding period (21 d) for histopathology. Fish used for histopathology were
201 euthanized using an overdose solution of MS-222, placed on ice, and shipped within 48 h to
202 Oregon State University's (OSU) Veterinary Diagnostic Laboratory (VDL) for histologic
203 examination. Fish that died during the experiment were fixed in 10% neutral buffer formalin as
204 described above and examined.

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

211 **Primary Stress Response**

212 Because of the potential for cortisol concentrations to decrease when a stressor subsides,
213 blood samples were collected from fish immediately upon arrival to the holding facility. Fish
214 (n=2) were anesthetized from each treatment replicate group using MS-222 prior to drawing 0.3-
215 1.0 ml blood from the heart using a 25G 2.54 cm needle and 3 ml syringe. Cardiac puncture was
216 necessary because the small size of the fish. Following blood sample collection, fish were
217 euthanized using an overdose solution of MS-222. To determine Yellow Tang ocean baseline
218 cortisol concentration, blood was collected from fish (n=4) underwater on SCUBA at capture
219 depth within 3 min of capture. Blood was injected into 3 ml vacutainer tubes with no additive
220 (Becton-Dickinson), placed on ice, and centrifuged at 3,000 rpm for 10 min <1 h later. Serum
221 supernatant was transferred to a clean vacutainer tube with no additive, placed on ice, and frozen

222 <1 h later for ≤ 40 d in a non-frostless freezer, and transported overnight on dry ice to the OSU
223 Department of Fisheries and Wildlife for analysis.

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

228 **Statistical Methods**

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

236 **Results**

237 **Mortality**

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

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

247 **Histopathology**

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

255 **Primary Stress Response**

256 The mean ocean baseline cortisol concentration was $8.9 \text{ ng}\cdot\text{ml}^{-1}$ ($SE = 4.96 \text{ ng}\cdot\text{ml}^{-1}$) and in some
257 cases was at or below the detection limit for the assay ($3.9 \text{ ng}\cdot\text{ml}^{-1}$). All treatment groups were
258 significantly elevated above the baseline cortisol concentration (all $p < 0.05$). Decompression
259 treatment significantly affected cortisol concentration (Two-way ANOVA: $F=4.26$; $df= 2,10$;
260 $p=0.03$). Ascent without decompression stops resulted in a significantly higher mean cortisol
261 concentration ($M=58.8 \text{ ng}\cdot\text{ml}^{-1}$, $SE=8.7 \text{ ng}\cdot\text{ml}^{-1}$) than ascent with many 15 min decompression
262 stops ($M=35.5 \text{ ng}\cdot\text{ml}^{-1}$, $SE=5.3 \text{ ng}\cdot\text{ml}^{-1}$), with neither treatment being significantly different
263 from ascent with one 45 min decompression stop ($M=35.2 \text{ ng}\cdot\text{ml}^{-1}$, $SE=4.3 \text{ ng}\cdot\text{ml}^{-1}$) (Figure 3).

264 Ascent without decompression stops produced the highest observed cortisol concentration
265 (101.49 ng•ml⁻¹), whereas the highest observed cortisol concentrations in fish subjected to one
266 and many decompression stops were 59.09 and 68.03 ng•ml⁻¹, respectively. While venting
267 resulted in higher mean cortisol concentration ($M=47.7$ ng•ml⁻¹, $SE=6.9$ ng•ml⁻¹) than the no
268 venting treatment ($M=38.2$ ng•ml⁻¹, $SE=4.3$ ng•ml⁻¹), this difference was not statistically
269 significant. In addition, there was no significant interaction between decompression treatment
270 and venting.

271 **Discussion**

272 With the objective of informing management on collection practices in the aquarium trade, our
273 study focused on the short- and long-term mortality of reef fish subjected to decompression and
274 venting as barotrauma prevention and mitigation practices, respectively. Overall, we found that
275 venting prevented immediate mortality in fish subjected to ascent without decompression stops.
276 Furthermore, we found only one case of a venting needle track, and the inflammation was
277 localized. There was no evidence of significant widespread tissue inflammation caused by
278 venting, or lesions linked to barotrauma immediately after collection, or following the long-term
279 21 d holding period. Finally, ascent to the surface significantly elevated serum cortisol above
280 baseline concentrations in fish at depth. Ascent without decompression stops resulted in
281 significantly higher serum cortisol concentrations than ascent with many stops. Venting did not
282 significantly affect cortisol concentrations, nor were there any significant interactions between
283 decompression and venting. In the following sections, we explain our results, suggest future
284 research recommendations, and discuss implications for fishery management.

285 **Mortality**

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

296 Additional factors that may influence post-collection mortality, but are outside the scope of this
297 study, include collection depth, body size, and species. We examined fish collected from 15-18
298 m depths, which is typical for the West Hawaii Yellow Tang fishery, though fishers do exceed
299 this range (i.e. ≥ 27 m) when targeting other species (Stevenson et al. 2011). At deeper depths,
300 the effects of decompression and venting may differ, and it is known that fish mortality and
301 occurrence of barotrauma increases with capture depth (Collins et al. 1999; St John and Seyers
302 2005; Hannah et al. 2008; Jarvis and Lowe, 2008; Campbell et al. 2010). Interviews with West
303 Hawaii fishers indicate that fish collected from >25 m require more decompression time and
304 venting while at depth, or several venting applications during ascent. Fishers have also
305 mentioned that larger fish exhibit more severe external barotrauma symptoms than smaller fish
306 of the same species, which is similar to findings in studies on deeper-dwelling food fishes
307 (Hannah et al. 2008; St John and Seyers 2005). Just as different deeper-dwelling food fish
308 species exhibit different responses to ascent rate (Hannah and Matteson 2007; Jarvis and Lowe

309 2008; Pribyl 2010), aquarium fish species reportedly react differently to ascent rate and venting.
310 These differences are likely caused by variation in body shape, tissue durability, and swim
311 bladder volume between species. Methods used by fishers reflect these species differences, with
312 practices such as performing venting on more delicate, soft-bodied fish like angelfish
313 (*Pomacanthidae*) underwater to prevent swim bladder expansion. Examining differences among
314 aquarium fish species of varying sizes and investigating the variety of techniques employed by
315 fishers during collection would provide further insight into the prevalence and effectiveness of
316 aquarium fish barotrauma prevention and mitigation methods.

317 **Histopathology**

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

327 Wound healing with no evidence of ongoing necrosis or inflammation, as seen in these fish,
328 indicates that the venting procedure does not pose a significant threat to fish survival post-
329 collection, nor does it cause significant sublethal effects. However, we caution that the fish in our
330 study were held in an aquaculture facility for 21 d without the additional handling and transport

331 stressors they would normally experience in the supply chain, thus potentially promoting
332 recovery from injuries inflicted during collection. It is possible that additional stressors of the
333 supply chain diminish the efficacy of venting in promoting long-term fish survival.

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

344 **Primary Stress Response**

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

353 appears that venting is a short-term stressor, but prevents mortality in fish subjected to ascent
354 without decompression stops.

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

364 **Implications for Management**

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

373 Though animal rights groups in Hawaii criticize venting, we did not find that it caused mortality
374 or sublethal injuries in Yellow Tang. Banning venting may increase mortality rates if fishers

375 implemented ascent without decompression. While opponents of venting have suggested that
376 slow decompression be used instead, the time required to properly decompress these fish is
377 economically prohibitive and impractical for fishers to implement.

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

383 **Acknowledgements**

384 We thank fishers in West Hawaii, especially Tyron Terrazono, Paul Masterjohn, and Scott
385 Brien for their time, cooperation, and support. Thanks to Todd Stevenson for project guidance;
386 Syd Kraul for use of his aquaculture facility; Meghan Dailer for her hospitality and
387 encouragement; Tony Spitzack, Cori Kane, and Molly Bøgeberg for project assistance; Dr. Jim
388 Beets and Caitlin Kryss of The University of Hawaii at Hilo's Marine Science Department, Dr.
389 Bill Walsh, Laura Livnat, and Kara Osada of the DAR for logistical support and project
390 guidance; Dr. Bob Jordan and the Kona Veterinary Service for supplies; Ian McComas for
391 centrifuge use, guidance, and his time; Dr. Carl Schreck and Julia Unrein at the OSU Department
392 of Wildlife and Fisheries Laboratory for cortisol analysis and guidance with sampling protocol;
393 Dr. Cheryl Schultz and many others for manuscript edits and suggestions.

394 **References**

- 395 Barton BA. 2002. Stress in fishes: A diversity of responses with particular reference to changes
396 in circulating corticosteroids. *Integrative and Comparative Biology* 42:517-525.
- 397 Barton BA, Iwama GK. 1991. Physiological changes in fish from stress in aquaculture with
398 emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases*
399 1:3-26.
- 400 Bruckner AW. 2005. The importance of the marine ornamental reef fish trade in the wider
401 Caribbean. *Revista de Biologia Tropica* 53:127–38.
- 402 Bruesewitz RE, Coble DW, Copes F. 1993. Effects of deflating the expanded swim bladder on
403 survival of burbot. *North American Journal of Fisheries Management* 13:346-348.
- 404 Campbell MD, Patino R, Tolan J, Strauss R, Diamond SL. 2010. Sublethal effects of catch-and-
405 release fishing: measuring capture stress, fish impairment, and predation risk using a
406 condition index. *ICES Journal of Marine Science* 67:513-521.
- 407 Capitini CA, Tissot BN, Carroll MS, Walsh WJ, Peck S. 2004. Competing Perspectives in
408 Resource Protection: The Case of Marine Protected Areas in West Hawai‘i. *Society and*
409 *Natural Resources* 17:763-778.
- 410 Cesar H, van Beukering P, Pintz S, Dierking J. 2002. Economic Valuation of the Coral Reefs of
411 Hawaii. *Pacific Science* 58(2):231-242.
- 412 Collins MR, McGover JC, Sedberry GR, Meister HS, Pardieck R. 1999. Swim bladder deflation
413 in black sea bass and vermilion snapper: potential for increasing postrelease survival.
414 *North American Journal of Fisheries Management* 19:828-832.
- 415 Donaldson EM. 1981. The pituitary-interrenal axis as an indicator of stress in fish. In: Pickering
416 AD ed. *Stress in Fish*. New York: Academic Press Inc., 11-47.
- 417 Gotshall DW. 1964. Increasing tagged rockfish (Genus Sebastodes) survival by deflating the
418 swim bladder. *California Fish and Game* 50:253-260.

- 419 Hall KC, Bellwood DR. 1995. Histological effects of cyanide, stress and starvation on the
420 intestinal mucosa of *Pomacentrus coelestis*, a marine aquarium fish species. *Journal of*
421 *Fish Biology* 47:438-454.
- 422 Hanawa M, Harris L, Graham M, Farrell AP, Bendell-Young LI. 1998. Effects of cyanide
423 exposure on *Dascyllus aruanus*, a tropical marine fish species: lethality, anesthesia and
424 physiological effects. *Aquarium Sciences and Conservation* 2:21-34.
- 425 Hannah RW, Matteson KM. 2007. Behavior of nine Pacific rockfish after hook-and-line capture,
426 recompression, and release. *Transactions of the American Fisheries Society* 136:24-33.
- 427 Hannah RW, Parker SJ, Matteson KM. 2008. Escaping the surface: the effect of capture depth on
428 submergence success of surface-released pacific rockfish. *North American Journal of*
429 *Fisheries Management* 28:694-700.
- 430 Jarvis ET, Lowe CG. 2008. The effects of barotrauma on the catch-and-release survival of
431 southern California nearshore and shelf rockfish (Scorpaenidae, *Sebastes* spp.). *Canadian*
432 *Journal of Fisheries and Aquatic Sciences* 65:1286-1296.
- 433 Jiménez del Río M, Ramazanov Z, García-Reina G. 1996. *Ulva rigida* (Ulvales, Chlorophyta)
434 tank culture as biofilters for dissolved inorganic nitrogen from fishpond effluents.
435 *Hydrobiologia* 326/327:61-66.
- 436 Jones RJ, Steven AL. 1997. Effects of cyanide on corals in relation to cyanide fishing on reefs.
437 *Journal of Marine and Freshwater Research* 48:517-522.
- 438 Jones RJ, Hoegh-Guldberg O. 1999. Effects of cyanide on coral photosynthesis: implications for
439 identifying the cause of coral bleaching and assessing the environmental effects of
440 cyanide fishing. *Marine Ecological Progress Series* 177:83-91.
- 441 Keniry MJ, Brofka WA, Horns WH, Mardsen JE. 1996. Effects of decompression and
442 puncturing the gas bladder on survival of tagged yellow perch. *North American Journal*
443 *of Fisheries Management* 16:201-206.
- 444 Kerr SJ. 2001. A review of “fizzing”- a technique for swim bladder deflation. Fish and Wildlife
445 Branch, Ontario Ministry of Natural Resources, Peterborough, Ontario. Available at

- 446 <http://www.rockymountainanglers.com/images/Studies%20Reports%20PDFs/ReviewOfF>
447 [izingTechniques.pdf](http://www.rockymountainanglers.com/images/Studies%20Reports%20PDFs/ReviewOfF). (accessed 5 September 2014).
- 448 Lauer NC. 2011, October 5. Supporters drown out opponents in testimony. *West Hawaii Today*.
449 Available at [http://kona.westhawaiiitoday.com/sections/news/local-news/fish-collecting-](http://kona.westhawaiiitoday.com/sections/news/local-news/fish-collecting-ban-reso-passes-council.html)
450 [ban-reso-passes-council.html](http://kona.westhawaiiitoday.com/sections/news/local-news/fish-collecting-ban-reso-passes-council.html). (accessed 5 September 2014).
- 451 LeGore RS, Hardin MP, and Ter-Ghazaryan D. 2005. Organization and operation of the marine
452 ornamental fish and invertebrate export fishery in Puerto Rico. *Revista de Biologia*
453 *Tropica* 53:145-153.
- 454 Nguyen V, Gravel M, Mapleston M, Hanson KC, and Cooke SJ. 2009. The post-release behavior
455 and fate of tournament-caught smallmouth bass after ‘fizzing’ to alleviate distended swim
456 bladders. *Fisheries Research* 96:313-318.
- 457 Parker SJ, McElderry HI, Rankin PS, and Hannah RW. 2006. Buoyancy regulation in two
458 species of nearshore rockfish. *Transactions of the American Fisheries Society* 135:1213-
459 1223.
- 460 Pribyl AL. 2010. A Macroscopic to Microscopic Study of the Effects of Barotrauma and the
461 Potential for Long-term Survival in Pacific Rockfish. D. Phil. Thesis, Oregon State
462 University.
- 463 Pyle R. 1993. Marine aquarium fish. Pacific Islands Forum Fisheries Agency, Honiara, Solomon
464 Islands. Available at
465 http://www.spc.int/DigitalLibrary/Doc/FAME/FFA/Reports/FFA_1992_055.pdf. (accessed
466 5 September 2014).
- 467 Randall JE. 1987. Collecting reef fish for aquaria. In Salvat B, ed. *Human Impacts on Coral*
468 *Reefs: Facts and Recommendations*. French Polynesia: Antenne Museum E.P.H.E., 29-
469 39.
- 470 Redding JM, Schreck CB, Birks E, Ewing RD. 1984. Cortisol and its effects on plasma thyroid
471 hormone and electrolyte concentrations in fresh water and during seawater acclimation in

- 472 yearling coho salmon, *Oncorhynchus kisutch*. *General and Comparative Endocrinology*
473 56:146-155.
- 474 Rhyne AL, Tlusty MF, Schofield PJ, Kaufman L, Morris JA, Bruckner AW. 2012. Revealing the
475 appetite and volume of the marine aquarium fish trade: the volume and biodiversity of
476 fish imported into the United States. *PLoS One* 7(5):e35808. doi
477 10.1371/journal.pone.0035808.
- 478 Roberts HE. 2010. Surgery and wound management in fish. In: Roberts HE, ed. *Fundamentals of*
479 *Ornamental Fish Health*. Ames, Iowa: Wiley-Blackwell, 185-196.
- 480 Rogers BL, Lowe CG, Fernandez-Juricich E, Frank LR. 2008. Utilizing magnetic resonance
481 imaging (MRI) to assess the effects of angling-induced barotrauma on rockfish
482 (Sebastes). *Canadian Journal of Fisheries and Aquatic Sciences* 65:1245-1249.
- 483 Rubec PJ, Cruz FP. 2005. Monitoring the chain of custody to reduce delayed mortality of net-
484 caught fish in the aquarium trade. *SPC Live Reef Fish Information Bulletin* 13:13-23.
- 485 Rubec PJ, Cruz FJ, Pratt V, Oellers R, McCullough B, Lallo F. 2001. Cyanide-free net caught
486 fish for the marine aquarium trade. *Aquarium Sciences and Conservation* 3:37-51.
- 487 Soares MC, Oliveira RF, Ros AFH, Grutter AS, Bshary R. 2011. Tactile stimulation lowers
488 stress in fish. *Nature Communications* 2: 534. doi: 10.1038/ncomms1547.
- 489 Stevenson TC, Tissot BN, Dierking J. 2011. Fisher behavior influences catch productivity and
490 selectivity in West Hawaii's aquarium fishery. *ICES Journal of Marine Science*
491 68(5):813-822.
- 492 Stevenson TC, Tissot BN. 2013. Evaluating marine protected areas for managing marine
493 resource conflict in Hawaii. *Marine Policy* 39:215-223.
- 494 St John J, Seyers CJ. 2005. Mortality of the demersal dhufish, *Glaucosoma hebraicum*
495 (Richardson 1845) following catch and release: The influence of capture depth, venting
496 and hook type. *Fisheries Research* 76:106-116.

- 497 Talbot R. 2012, January 19. Senate Bills Call for Total Ban on Hawaiian Fishery: Open Season
498 on Marinelife Collectors. *Coral: The Reef and Marine Aquarium Magazine Newsletter*.
499 Available at [http://www.coralmagazine-us.com/content/senate-bills-call-complete-ban-](http://www.coralmagazine-us.com/content/senate-bills-call-complete-ban-sale-hawaiian-aquatic-life)
500 [sale-hawaiian-aquatic-life](http://www.coralmagazine-us.com/content/senate-bills-call-complete-ban-sale-hawaiian-aquatic-life). (accessed 5 September 2014).
- 501 Tissot BN. 2005. Integral marine ecology: community-based fishery management in Hawaii.
502 *World Futures: General Evolution Research Group* 61:79-95.
- 503 Tissot BN, Hallacher LE. 2003. Effects of aquarium collectors on coral reef fishes in Kona,
504 Hawaii. *Conservation Biology* 17:1759-1768.
- 505 Tissot BN, Best BA, Borneman EH, Bruckner AW, Cooper CH, D'Agnes H, Fitzgerald TP,
506 Leland A, Lieberman S, Amos AM, Sumaila R, Telecky TM, McGilvray F, Plankis BJ,
507 Rhyne AL, Roberts GG, Starkhouse B, Stevenson TC. 2010. How U.S. Ocean Policy and
508 Market Power Can Reform the Coral Reef Wildlife Trade. *Marine Policy* 34:1385–1388.
- 509 Vandermeulen H, Gordin H. 1990. Ammonium uptake using *Ulva* (Chlorophyta) in intensive
510 fishpond systems: mass culture and treatment of effluent. *Journal of Applied Phycology*
511 2:363-374.
- 512 Walsh WJ, Cotton SP, Dierking J, Williams ID. 2004. Status of Hawaii's Coastal Fisheries in the
513 New Millennium (The commercial marine aquarium fishery in Hawai'i 1976–2003). In:
514 Friedlander AM, ed. Hawaii Chapter, Honolulu: American Fisheries Society, 132-159.
- 515 Williams ID, Walsh WJ, Claisse JT, Tissot BN, Stamoulis KA. 2009. Impacts of a Hawaiian
516 marine protected area network on the abundance and fishery sustainability of the Yellow
517 Tang (*Zebrasoma flavescens*). *Biological Conservation* 142:1066-1073.
- 518 Wilde GR. 2009. Does venting promote survival of released fish? *Fisheries* 34(1):20-34.
- 519 Wintner R. 2010, August 12. "Maui County Council Ordinance Curbs Reef Extraction for
520 Aquarium Trade." *The Huffington Post*. Available at
521 [http://www.huffingtonpost.com/robert-wintner/maui-county-council-](http://www.huffingtonpost.com/robert-wintner/maui-county-council-ordina_b_674889.html)
522 [ordina_b_674889.html](http://www.huffingtonpost.com/robert-wintner/maui-county-council-ordina_b_674889.html). (accessed 5 September 2014).

523 Wintner R. 2011, January 27. "The Second Foot Falls on the Aquarium Trade in Maui County."
524 *The Huffington Post*. Available at [http://www.huffingtonpost.com/robert-wintner/a-](http://www.huffingtonpost.com/robert-wintner/a-splash-heard-round-the-_b_812604.html)
525 [splash-heard-round-the-_b_812604.html](http://www.huffingtonpost.com/robert-wintner/a-splash-heard-round-the-_b_812604.html). (accessed 5 September 2014).

526 Wood E, 2001. Collection of Coral Reef Fish for Aquaria: Global Trade, Conservation Issues,
527 and Management Strategies. Marine Conservation Society, UK, 80pp. Available at
528 http://www.eldis.org/go/home&id=11010&type=Document#.VAqh_Kgqils. (accessed 5
529 September 2014).

530

531

532

533

534

535

536

537

538

539

540

541

542

543

544

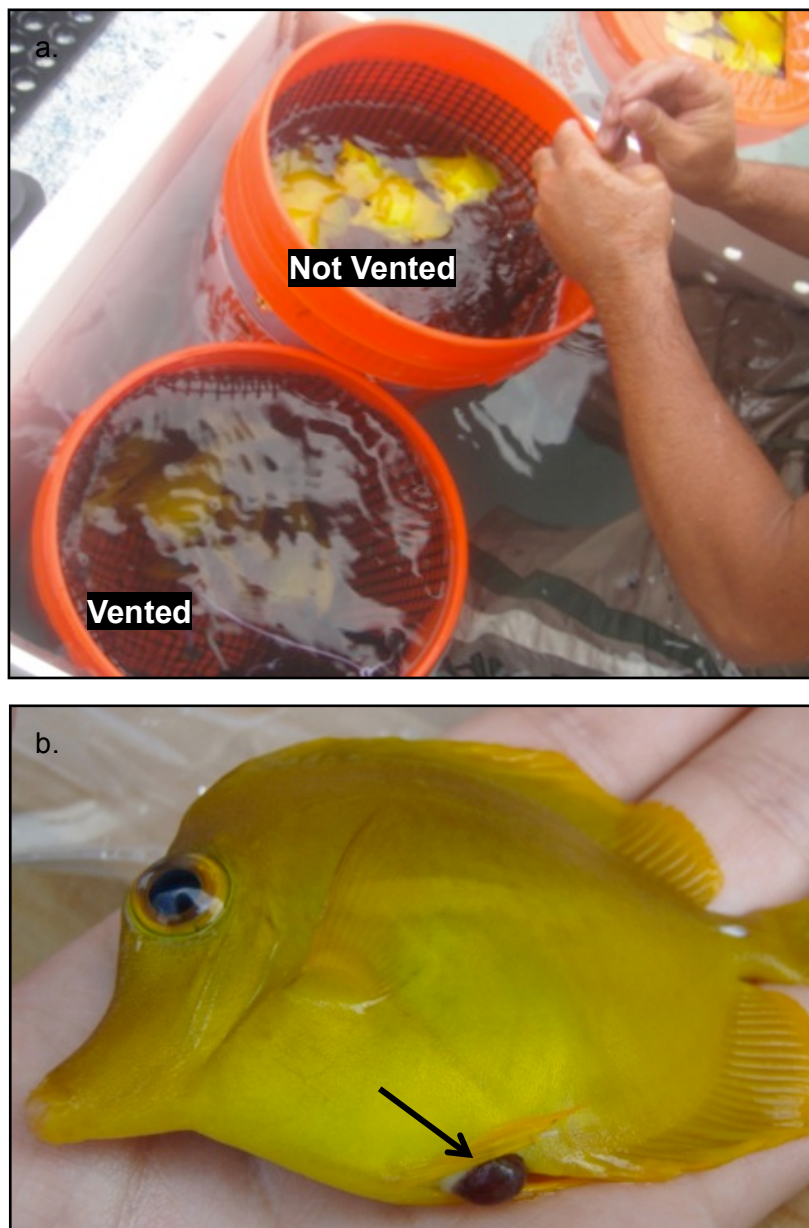


Figure 1: Barotrauma signs observed in Yellow Tang following collection: (a) positive buoyancy before venting and neutral to negative buoyancy following venting (b) intestinal protrusion from the cloaca.

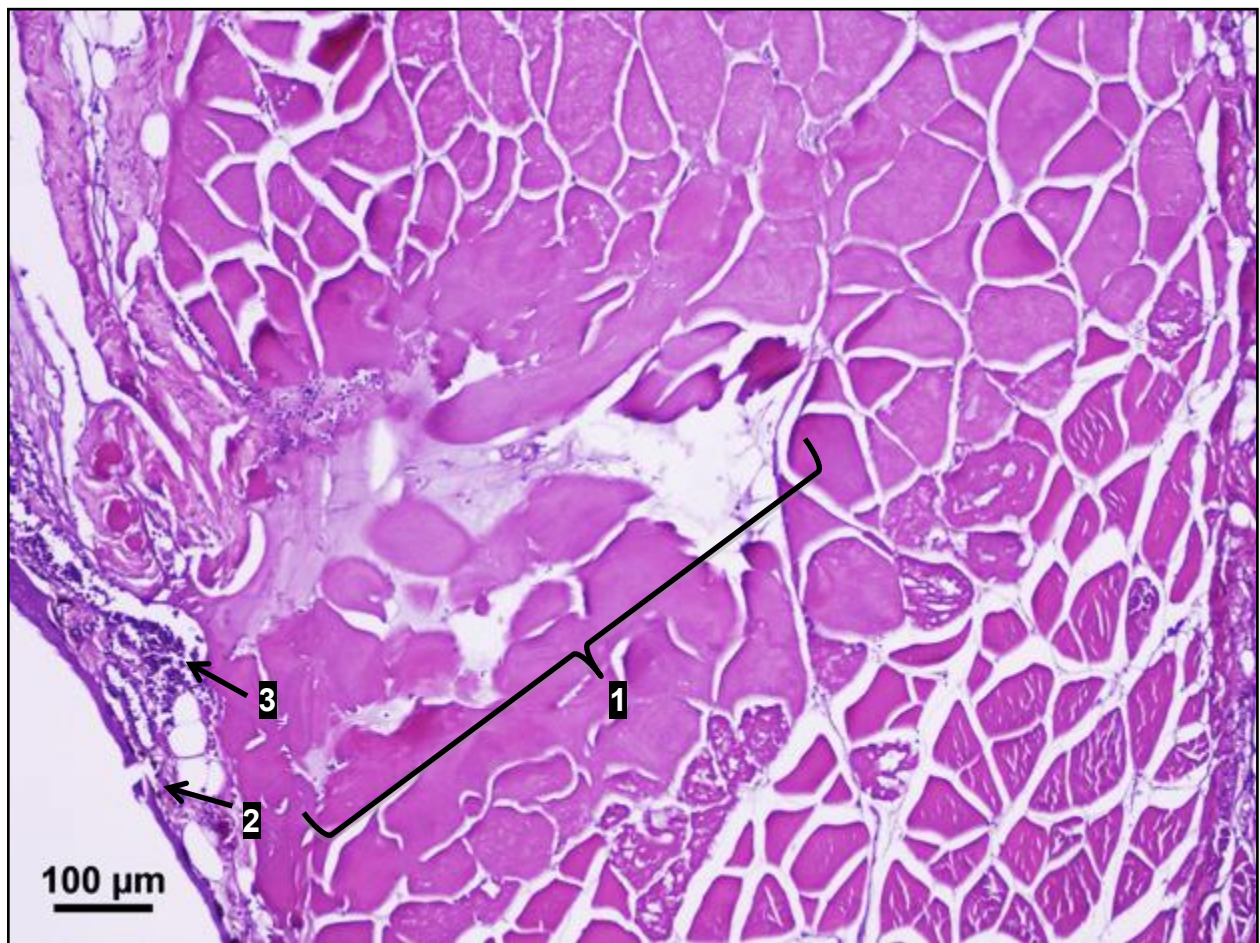


Figure 2: Histological section of needle track in a Yellow Tang subjected to venting showing muscle cell necrosis, edema, and neutrophilic inflammation, at 10x magnification. (1) Needle track, (2) needle entry through coelomic cavity, (3) neutrophilic inflammatory response.

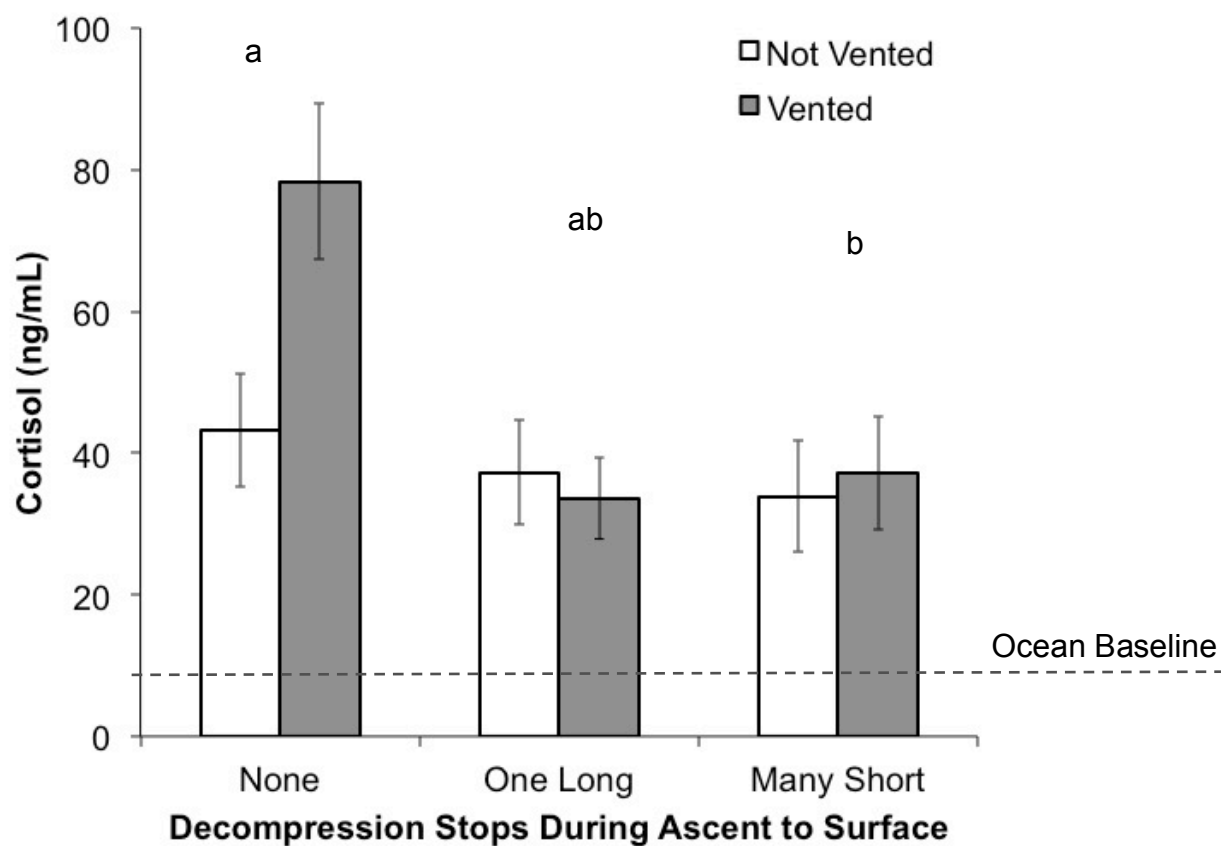


Figure 3: Cortisol concentration (mean \pm SE) by each treatment. Letter groups represent Tukey's multiple range test results comparing means between decompression treatments. All treatment groups were significantly elevated above the ocean baseline concentration of $8.9 \text{ ng}\cdot\text{ml}^{-1}$.