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The effect of rapid decompression on barotrauma and survival rate in swallowtail seaperch (*Anthias anthias*): Defining protocols for mitigating surfacing mortality

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ABSTRACT

Swallowtail seaperch, *Anthias anthias,* is a popular fish in the public aquaria industry worldwide, but is subject to barotrauma and high mortality rates if an appropriate decompression profile is not used. Here, we analyze behavioral response to pressure reductions in swallowtail to define protocols for mitigating surfacing mortality. Four different pressure reduction rates were tested (15%, 25%, 35%, and 45%) in several lifting steps from an initial depth of 30 m. Decompression using this procedure was done with 12 and 24 h acclimation duration at each step allowing fish to recover from the pressure reduction. Fish condition was assessed based on swimming behavior, immediately after each new pressure reduction also after each acclimation time. Additionally, fish condition was monitored in a post-decompression trial for 14 days. During decompression, both conditions - initial and final - showed statistically significant differences in the reduction rates tested but showed no differences in acclimation times, and no interaction of the two factors. Neither pressure nor acclimation time affected the condition of the animal in post-decompression trials. Ascension steps near the surface are associated with larger decreases in neutrally buoyant fish compared to deeper decompression steps. Close monitoring of the effect of decompression on *A. anthias* in the control group, showed that a 29% reduction in pressure could indicate an approximate value of the free vertical range of this species, while swimbladder rupture can occur between 63 and 70% of pressure reduction. The optimal protocol for mitigating surfacing mortality combines two decompression profiles used in this experiment with a total duration of 84 h and comprising 4 lifting steps. The protocol developed to mitigate surfacing mortality was designed for conditions where oceanic cages or containers can be lifted gradually.

1. Introduction

Fish barotrauma is associated with rapid decompression that may occur during fishing operations (Wilson and Burns, 1996; Burns and Restrepo, 2002; Lenanton et al., 2009; Butcher et al., 2012), lifting fish cultured in oceanic sea-cages (Korsøen et al., 2010; Ferter et al., 2015), or fish passing hydroelectric facilities (Colotelo et al., 2012; Richmond et al., 2014). It is defined as a physical trauma resulting from a rapid decrease in ambient pressure, and is common amongst fish captured from depth (Rummer and Bennett, 2005; Nichol and Chilton, 2006).

Barotrauma is common in fish with a closed swimbladder (physoclistous), which regulates buoyancy over depth changes by secreting and reabsorbing swimbladder gases (Jones, 1952; Parker et al., 2006). As gas secretion and reabsorption are relatively slow processes, the "free vertical range" that the fish can swim and compensate is restricted to the extent and speed of the changes in depth and time taken to adjust to a new pressure (Jones and Scholes, 1985; Stensholt et al., 2002). In physoclistous fish, a rapid compression or decompression of individuals can be harmful or lethal, as a result of the rapid compression or expansion of gases (Burns and Restrepo, 2002; Hannah and Matteson, 2007; Korsøen et al., 2010).

Fish suffering from decompression barotrauma usually exhibit characteristic symptoms (Rummer and Bennett, 2005). Expanded gases can cause swimbladder distension or rupture which, in some cases,

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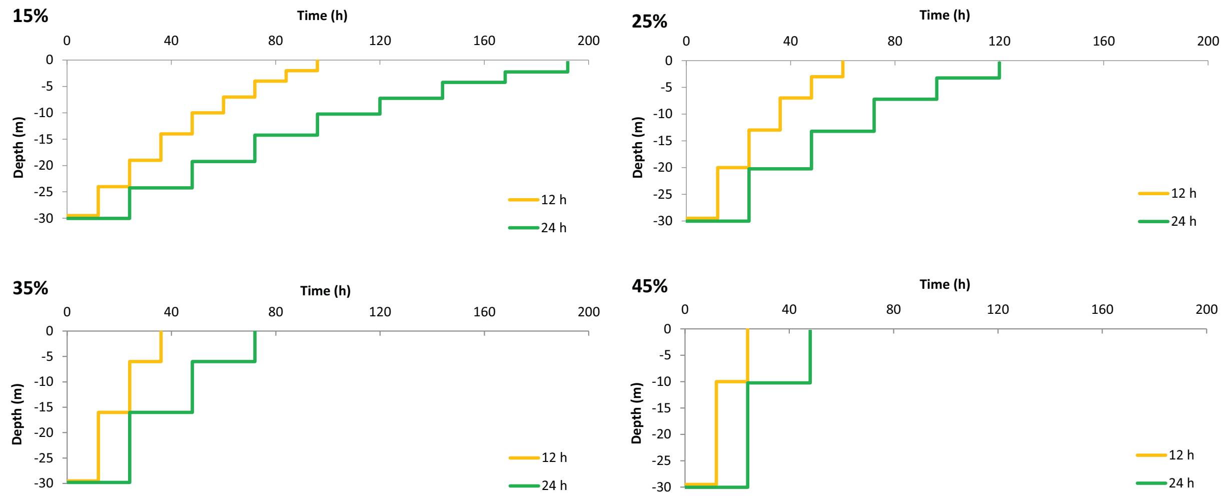

Fig. 1. Decompression experimental design used in *A. anthias* surfacing, representing the sequence of depth reduction at different pressure change rates and acclimation times. All 8 treatments started from an initial depth of 30 m. 12 and 24 h indicate the number of waiting hours between ascension steps.

includes compression injuries to organs, eversion of the esophagus or stomach, or intestinal protrusions from the anus. Exposure to barotrauma can also cause exophthalmia, embolism and hemorrhaging in almost all body tissues, such as gills, heart, liver and brain (Gitschlag and Renaud, 1994; Rummer and Bennett, 2005; Parker et al., 2006; Hannah and Matteson, 2007; Gravel and Cooke, 2008; Jarvis and Lowe, 2008; Wilde, 2009).

Several studies have been conducted in order to understand the range of depths and pressure changes relative to neutral buoyancy in physoclistous fish. Godù and Michalsen (2000) and Stensholt et al. (2002) indicate that the free vertical range of cod, *Gadus morhua*, corresponds to no more than a 50% pressure reduction in depth, relative to neutral buoyancy. Korsøen et al. (2010) recommend that lifting steps of cages in farmed cod should be lower than 40%, while maintaining a slower rate, to enable the recovery of cod. Although barotrauma is a common issue in the capture of live fish for the public aquaria industry, it has been rarely addressed. Barotrauma has prevented deep water fish to be exhibited in public aquaria and therefore limiting environmental awareness activities related to deep-sea ecosystems. Holding live fish in sea cages and performing optimal decompression profiles may help overcoming barotrauma problems in fish captured in deeper waters and allow deep water fish to be exhibited in public aquaria.

The swallowtail seaperch, *Anthias anthias* (Linnaeus 1758), is a physoclistous fish very popular in the public aquaria industry worldwide, mainly because of its unique appearance and striking coloration. This species displays gregarious behavior, inhabiting rocky substrates and submarine caves commonly at depths between 30 and 200 m (Paglialonga et al., 2001; Micarelli and Barlettani, 2005; Espino et al., 2007), and is subject to barotrauma and high mortality rates associated with rapid decompression if an appropriate decompression profile is not used (Paglialonga et al., 2001; Micarelli and Barlettani, 2005). Internal or external signs of barotrauma have not yet been described for any Anthinidae species and reliable protocols for mitigating ascendance injury and mortality have not been developed.

Therefore, the objective of this study was to develop a protocol for mitigating surfacing mortality in swallowtail seaperch, while assessing its resilience to changes in pressure during ascendance to the surface. We aimed specifically at *i)* assessing fish condition state of swallowtail seaperch subject to different pressure changes and acclimation times during decompression; *ii)* quantifying post-decompression fish condition state and recovery rates; and *iii)* determine an optimum decompression protocol that reduces mortality and increases long-term survival of swallowtail seaperch.

2. Materials and methods

2.1. Study location and fish collection

This study was conducted in Faial Island, Azores in 2012. Capture and decompression trials were conducted in Baía da Garça (38°31′N; 028°37′W) due to its proximity to the harbor and easy access by boat. This bay is relatively sheltered throughout the year and large schools of *A. anthias* are usually found. Fish were collected by a team of 3 to 5 divers at depths varying from 33 to 40 m. To do this, a knotless seine net (2.5 cm stretched mesh size) was adapted to be operated underwater. This stationary net had a floatline along the top and a leadline with sinkers along the base, keeping the floats from lifting the net from the bottom and forming a vertical wall with approximately 4 m wide x 2.5 m high. When schools of *A. anthias* were detected, the fish were huddled and driven by the divers towards the net. Fish were then captured with hand nets, handled very gently and placed in custom designed decompression containers. These consisted of 15 L polypropylene cylindrical shape containers, with an acrylic window on top to allow the observation of fish behavior during decompression. The containers had holes around which allowed for water flow and keeping the pressure inside and outside the container equal. Fish densities were kept constant at 5 fish per container whenever possible. In total, 83 *A. anthias* were collected.

2.2. Decompression experimental design

After collection, *A. anthias* were maintained in decompression containers at 30 m depth for at least 24 h to allow them to recover from any possible stress caused by capture. A decompression line was set consisting of a polypropylene cable anchored at 30 m depth with a dead weight of approximately 100 kg connected to a subsurface buoy that ensured the cable was permanently under tension. The cable was marked at the multiple depths at which different treatments would make a decompression stop. At each decompression step containers were well secured with strong elastic straps.

To test behavioral responses and survival rates due to pressure reduction, a series of container lifting steps were conducted. Four different treatments (i.e. changes in pressure rates) were tested, corresponding to 15%, 25%, 35% and 45% reductions in pressure from the initial depth of 30 m (i.e. 4 ATA, absolute atmospheres) or previous lifting step. The number of lifting steps and at which depth they occurred was variable and is illustrated in Fig. 1. Decompression using this procedure was done with two different acclimation durations between each pressure change: 12 and 24 h (Fig. 1). Each lifting to a new depth was conducted by the same diver at a standardized ascension rate of 4 m·min^{-1}. In summary, 8 different treatments of percentage of pressure reduction x acclimation time were tested: 15% x 12 h (n = 10), 15% x 24 h (n = 10), 25% x 12 h (n = 14), 25% x 24 h (n = 5), 35% x 12 h (n = 10), 35% x 24 h (n = 13), 45% x 12 h (n = 5), and 45% x 24 h (n = 11). Uneven sample sizes resulted from difficulties in capturing a sufficient number of fish in each fishing event.

2.3. Assessing fish condition state

Assessment of the behavior of the animals based on swimming speed, tail beat and swimming angle, were used to assign a fish condition state. The fish condition states used in this study were:

i. Moribund with positive buoyancy: fish floating at the surface or top of the decompression container with apparent tight abdomen symptom and lacking the ability to swim to the bottom or sink;
ii. Positively buoyant: fish with downward compensatory swimming (head down/caudal fin up) displaying accelerated and powerful tail-beating and swimming speed;
iii. Neutrally buoyant: fish that were able to maintain a horizontal position with minimal movement;
iv. Negatively buoyant: fish displaying compensatory swimming (head up/caudal fin down) with accelerated tail-beating in order to keep off the bottom;
v. Moribund with negative buoyancy: fish on the bottom showing very little activity and with no reaction of stimulus;
vi. Dead.

Neutrally buoyant fish (iii) were considered to be in the best condition, immediately followed by positively buoyant fish (ii), which were believed to have the swimbladder slightly inflated but with a high probability for gas resorption. Negatively buoyant fish (iv) were believed to have suffered rupture of the swimbladder with moderate probability for recovering. Moribund with positive and negative buoyancy (i and v) were believed to be in the worst condition state and with the lowest probability for surviving (Alexander, 1966; Stevens, 2011).

2.4. Assessing fish condition during decompression

Fish condition state was evaluated during the ascendance to surface i) immediately after each lifting step (C_i), to identify the immediate response to pressure reduction; and ii) after two different acclimation durations (C_f) following each pressure reduction, to identify the recovery response after 12 and 24 h.

The control group was brought to the surface directly from 30 m depth corresponding to a 75% pressure reduction. This procedure was performed by the same diver and also at the standardized rate of 4 m·min-1. A diver's safety stop at 5 m for 3 min was always respected. During the ascension of the control group, the diver observed the depths at which: (i) individuals went from neutral to positively buoyant; (ii) intestinal eversion occurred; (iii) swim bladder rupture occurred as observed by the release of gas bubbles from the anus. All fish were brought to the surface individually to allow for easier observations (n = 5).

2.5. Assessing fish condition during post-decompression

Once at the surface, the animals were kept on land-based tanks for approximately 14 days. During this period, all fish were monitored and assessed closely, to identify behavioral effects and survival rates: (i) short-term; measured after 24 h ($C_{24\ h}$) at surface; (ii) medium term; measured after 72 h ($C_{72\ h}$) at the surface (iii) long-term; measured after 14 days ($C_{14\ d}$). Similarly to the decompression evaluation, post-decompression evaluation used the same 6 fish condition state (see above).

A. anthias were held in 1200 L cylindrical polyethylene tanks, each divided in 8 equal sections containing the different decompression treatments with the respective replicates as well as the control group. Initial fish densities were 5 fish per tank section in most cases. The tanks were equipped with mechanical filtration in a semi-closed recirculating system, with 100% water changes daily. New water was pumped directly from the nearby shore, as the staging facility was located inside Horta's harbor. Water from tanks parameters were monitored every day to ensure optimal water quality: temperature (20.5 ± 2.3 °C; range 17.1–24.7 °C), ammonia (0.001 mg L^{-1}; range 0–0.027 mg L^{-1}), pH (8.1 ± 0.1; range 8.0–8.2), dissolved oxygen (91 ± 7%; range 60–100%) and salinity (36 PSU). Twenty-four hours after arrival fish were fed with frozen shrimp and food pellets. Food was then offered to satiation every second day. Fish were provided with PVC pipes with multiple diameters, positioned in the bottom of the tank, to provide shelter and reduce stress.

2.6. Data analysis

Data did not meet the normality and homoscedasticity assumptions, even after transformation, and a PERMANOVA multivariate analysis of variance test (unrestricted permutation of raw data) was therefore applied (Anderson et al., 2008). This statistical analysis is a powerful non-parametric approach that uses a permutational technique to enable significance tests for small sample sizes to be conducted (Walters and Coen, 2006) and was used to test the effect of pressure reduction and acclimation time on the fish condition state and recovery rates. The analyses were conducted using the software PRIMER 6 & PERMANOVA using a resemblance matrix based on Euclidean and treatments as fixed effects. The PERMANOVA was run using 9999 permutations to produce p values using the Monte Carlo (MC) method. When the main test produced a significant result ($p < .05$), a pairwise test was conducted to identify the individual differences between treatments.

3. Results

3.1. Assessing fish condition state during decompression

Overall, the average proportion of neutrally buoyant fish immediately after each pressure reduction was higher at lower pressure change rates and longer acclimation duration (Fig. 2a). However, the PERMANOVA with Monte Carlo test showed significant differences between pressure changes (p (MC) < 0.0001), no significant differences between acclimation time (p (MC) = 0.9776), and the interaction of the two factors was not significant (p (MC) = 0.067) Table 1.

After acclimation time at each depth, the proportion of neutrally buoyant fish was also higher at lower pressure change rates but did not show a marked trend with acclimation duration (Fig. 2b). The PERMANOVA with Monte Carlo test showed significant differences between pressure changes (p (MC) = 0.035), no significant differences between acclimation time (p (MC) = 0.556), and the interaction of the two factors was not significant (p (MC) = 0.367). The higher proportion of neutrally buoyant fish arriving to the surface occurred in 24 h acclimation durations in the 15%, 25%, 35% and 45% pressure change treatments with 0.90, 1, 0.85 and 0.91 of neutrally buoyant fish, respectively. The control group, subjected to a 75% pressure reduction, revealed that only 0.40 of fish arrived to the surface neutrally buoyant. The 12 h acclimation period was, on average, sufficient for the recovery of fish subject to a pressure change of 15%, 25% and 35% but apparently not enough for those individuals subjected to a 45% pressure change.

During the decompression steps, as fish were being lifted, the proportion of neutrally buoyant decreased as the number of positively buoyant fish increased, which is caused by the reduction in pressure

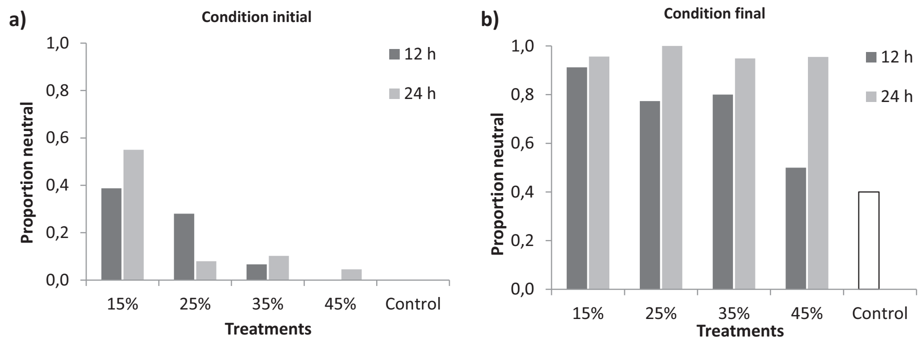

Fig. 2. Average proportion of neutrally buoyant *A. anthias* during decompression stages. a) Condition initial (C_i) immediately after each pressure reduction and b) Condition final (C_f) after acclimation time at each depth. The values were obtained by an average of all C_i and C_f in the corresponding steps of the treatments.

Table 1
PERMANOVA results of the effects of pressure reduction and acclimation time in the condition of *A. anthias* during decompression (C_i and C_f) and during post-decompression ($C_{24\ h}$, $C_{72\ h}$ and $C_{14\ d}$).

Variable	Source	df	SS	MS	Pseudo-F	p (MC)
C_i	Pressure	3	9442	3147	18,020	**0,0001**
	Duration	1	$1337*10^{-4}$	$1337*10^{-4}$	$7{,}66*10^{-4}$	0,9776
	Pressure x Duration	3	1259	0,420	2402	0,0666
C_f	Pressure	3	0,974	0,325	2868	**0,0353**
	Duration	1	$3954*10^{-2}$	$3954*10^{-2}$	0,349	0,5565
	Pressure x Duration	3	0,360	0,120	1060	0,3673
$C_{24\ h}$	Pressure	3	0,356	0,119	0,690	0,5730
	Duration	1	1268	1268	7353	**0,0097**
	Pressure x Duration	3	0,429	0,143	0,829	0,4774
$C_{72\ h}$	Pressure	3	0,189	$6316*10^{-2}$	0,432	0,7217
	Duration	1	$9{,}56*10^{-2}$	$9556*10^{-2}$	0,654	0,4162
	Pressure x Duration	3	$2224*10^{-2}$	$7412*10^{-3}$	$5{,}0697*10^{-2}$	0,9850
$C_{14\ d}$	Pressure	3	0,116	$3882*10^{-2}$	0,279	0,8442
	Duration	1	$5768*10^{-2}$	$5768*10^{-2}$	0,415	0,5253
	Pressure x Duration	3	$3{,}7253*10^{-2}$	$1241*10–^{2}$	$8{,}9275*10^{-2}$	0,9647

p (MC): p value based on Monte Carlo random draws. Significant p values are marked in bold.

(Figs. 3a - h). After acclimation (12 h or 24 h) to each new depth, the proportion of neutrally buoyant fish generally increased as a result of fish adapting to their new ambient pressure (Figs. 3a - h). These results revealed that in the four pressure reductions tested, the proportion of neutral fish was higher in the deeper lifting steps, decreasing in the subsequent decompression stages, especially above 12 m. Moreover, it was observed a trend for the increase of the proportion of neutral fish between 12 h and 24 h acclimation with the same pressure reduction, showing a better condition of fish after 24 h acclimation time.

The 15% treatment group data, however, showed some unexpected results, particularly in steps 24 m depth and 19 m depth, where the proportion of neutrally buoyant fish was higher in the 12 h groups than in its 24 h equivalent (Fig. 3a and e). Another unexpected result was the lower proportion of neutral individuals in the 25% x 12 h decompression step at 7 m, when compared to 3 m (Fig. 3 a). In this case, one individual died unexpectedly and was subsequently subject to necropsy. Death was attributed to infections resulting from fin and scale loss, with multiple lesions attributed to contact with the traps and was not directly attributed to decompression illness. This loss most likely derived from the collection procedure itself. Two other deaths occurred during the decompression trials, both of which related to barotrauma. One at the second lift of the 35% x 12 h treatment and another one during the third lift of the 25% x 12 h treatment. The former death was also necropsied, and barotrauma symptoms were identified, such as tight abdomen and several internal hemorrhages. The latter death displayed, upon necropsy, formation of gas bubbles in the pharyngo-cleithral membrane.

3.2. Assessing fish condition state post-decompression trials

The fish condition state post-decompression trials were assessed in the short-term (24 h), medium term (72 h) and long-term (14 days). Twenty four hours after arriving to the surface, the proportion of neutrally buoyant *A. anthias* post-decompression trial, was higher for all 24 h treatments when compared to the 12 h treatments (Fig. 4). There was no clear trend on the effect of the pressure change in the condition of fish 24 h after arriving to the surface. Accordingly, the PERMANOVA with Monte Carlo test showed significant differences between acclimation time ($p\ (MC) < 0.010$), but no significant differences between pressure changes ($p\ (MC) = 0.573$), or the interaction of the two factors ($p\ (MC) = 0.477$).

Medium term monitoring indicated higher proportion of neutrally buoyant fish in 35% x 24 h, 15% x 24 h and 35% x 24 h treatments (Fig. 4). However, PERMANOVA with Monte Carlo test showed no significant differences between acclimation time ($p\ (MC) = 0.416$), pressure changes ($p\ (MC) = 0.721$), or the interaction of the two factors ($p\ (MC) = 0.985$).

Long term monitoring after 14 days demonstrated a similar scenario, where the same higher proportions of neutrally buoyant fish were observed (Fig. 4). Similarly, the PERMANOVA with Monte Carlo test showed no significant differences between acclimation time ($p\ (MC) = 0.525$), pressure changes ($p\ (MC) = 0.844$), or the interaction of the two factors ($p\ (MC) = 0.965$). The general condition states of *A. anthias* after 14 days of acclimation were generally very good in all treatments. The results also showed that, with the exception of 25% pressure reduction rate, all treatments had a higher proportion of neutrally buoyant fish in 24 h acclimation time. The 25% treatment was the only displaying an equal proportion of neutrally buoyant fish for both 12 h and 24 h. The control group after 14 days revealed the lowest proportion neutrally buoyant fish (0.60; Fig. 4). Only one mortality occurred during the post-decompression trial and corresponded to the 15% x 12 h group. Upon necropsy, tight abdomen symptom was identified with the swimbladder also over inflated and a formation of a red ring around the anus. Despite death occurring at the surface, this was an obvious consequence of decompression illness.

3.3. Evaluation of the control group

The evaluation of the control group showed that the average of depth at which fish became positive buoyant was at 18.4 ± 1.4 m (Fig. 5), corresponding to a 29% pressure reduction. Intestinal protrusion occurred in four fish at 4.7 ± 2.1 m (Fig. 5 a - d), corresponding to a 63% pressure reduction, from initial depth. Additionally,

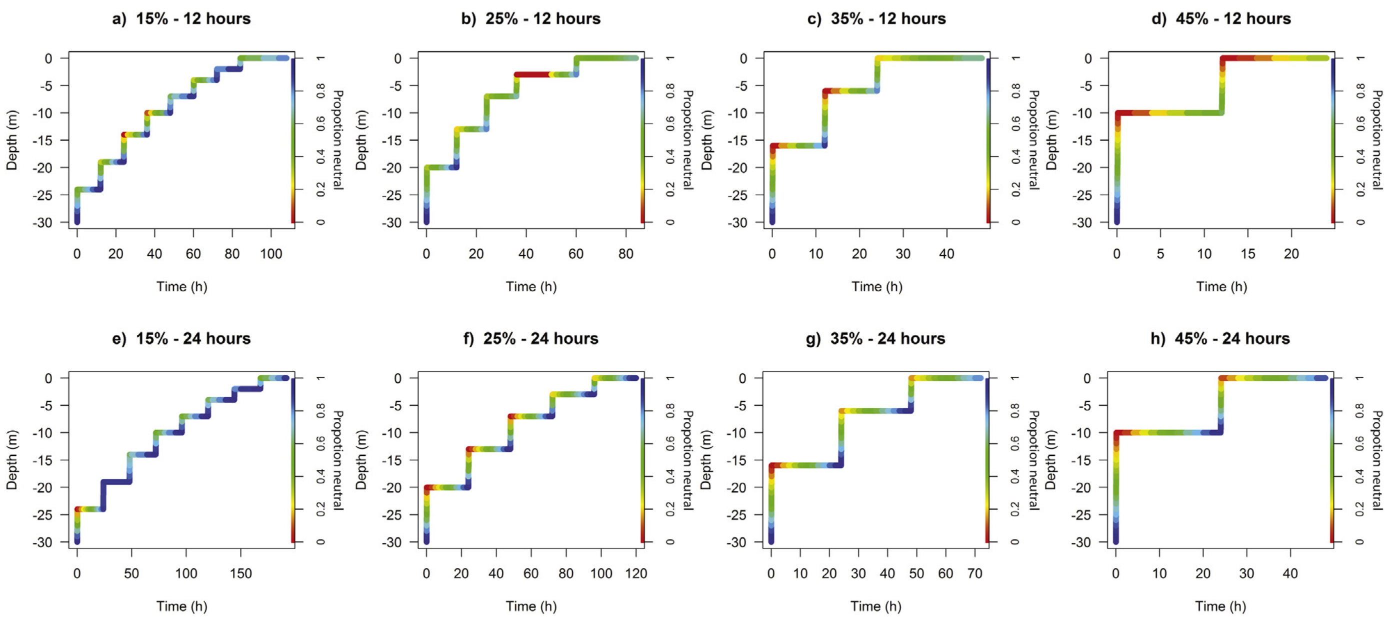

Fig. 3. Proportion of neutrally buoyant fish during decompression steps; a – d) for the four pressure reductions rates with 12 h acclimation time; e - h) for the four pressure reductions with 24 h acclimation time. Red indicates lower proportion of neutrally buoyant fish or bad condition state while blue indicate higher proportions of neutrally buoyant fish or good condition state. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

swimbladder ruptures were observed in four fish, occurring at 1.9 ± 0.87 m (Fig. 5 a - d), corresponding to a 70% pressure reduction from initial depth. Three fish were moribund positive when arrived at the surface, with swollen abdomen symptom while floating at the top of the decompression container (Fig. 5 b, c, d). The other two individuals displayed typical positive buoyant behavior with a fast-compensatory downward swimming. The post-decompression trial revealed that after 24 h in the tanks there were four fish positively buoyant and one with negative buoyancy. After 72 h two fish were negatively buoyant, and three fish apparently regained their neutral buoyancy.

4. Discussion

Although this study encompasses some limitations, such as reduced (n = 83) and uneven samples size (not always 15 per treatment), or the reduced number of acclimation times (12 and 24 h), it successfully addressed the effect of different decompression profiles on barotrauma and behavior responses to pressure changes in *A. anthias.* Changes in pressure rates tested in this protocol were within the ranges at which some physoclistous fish can recover and restore neutral buoyancy (Godù and Michalsen, 2000; Stensholt et al., 2002; Korsøen et al., 2010). It is known that the capacity of a physoclistous fish to adapt to a range of depths is mainly related to its natural behavior, in terms of daily vertical migrations, as the swimbladder can restrict these movements (Jones and Scholes, 1985; Pribyl et al., 2009).

As with other species, e.g. cod *G. morhua* (Jones and Scholes, 1985; Korsøen et al., 2010), visually examining the behavioral responses in *A. anthias* subjected to pressure reduction, proved to be an effective method. Similar behavior was observed in positively buoyant fish during the decompression and post-decompression trials. After lifting events, positive buoyancy was compensated with a downward fast compensatory swimming, with continuous strong tail beats and showing a head down/tail up position relative to horizontal. In the post-decompression trial, positively buoyant fish were constantly looking for shelter inside PVC tubes to avoid emerging and reduce the energy loss caused by constant downward swimming.

Although the experimental protocol wasn't designed to understand the exact duration necessary to adapt to a certain pressure change, our results suggest that 24 h acclimation time in a new depth can benefit fish health, compared to an acclimation time of 12 h. In all four pressure reduction rates tested, the proportion of neutral individuals at the surface was always higher in the 24 h acclimation time.

The cumulative effect observed, where in deeper lifting steps the proportion of neutrally buoyant fish was higher compared to shallower steps, could be explained by the pressure that occurs in deeper water compared to those in shallower water. Jones (1952) and Fänge (1983) described similar results for cod, *Gadus morhua*, where the rate of gas resorption increased with the pressure to which the fish were adapted from. These results indicate that in different magnitude ascensions, involving the same proportional pressure reductions, the time taken to

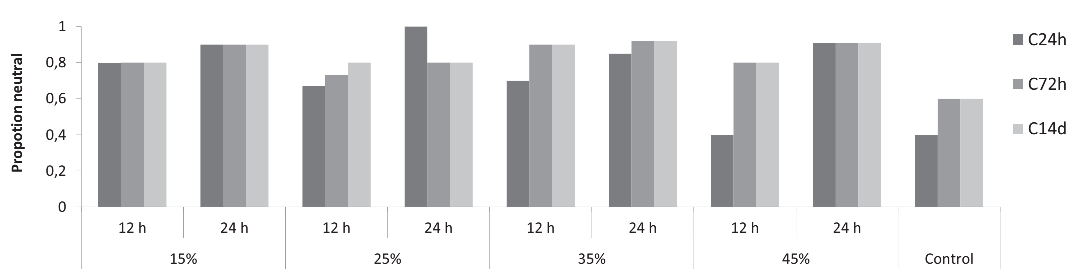

Fig. 4. Proportion of neutrally buoyant *A. anthias* during post-decompression trials assessed in the short-term (C_{24h}), medium term (C_{72h}), and long-term (C_{14d}).

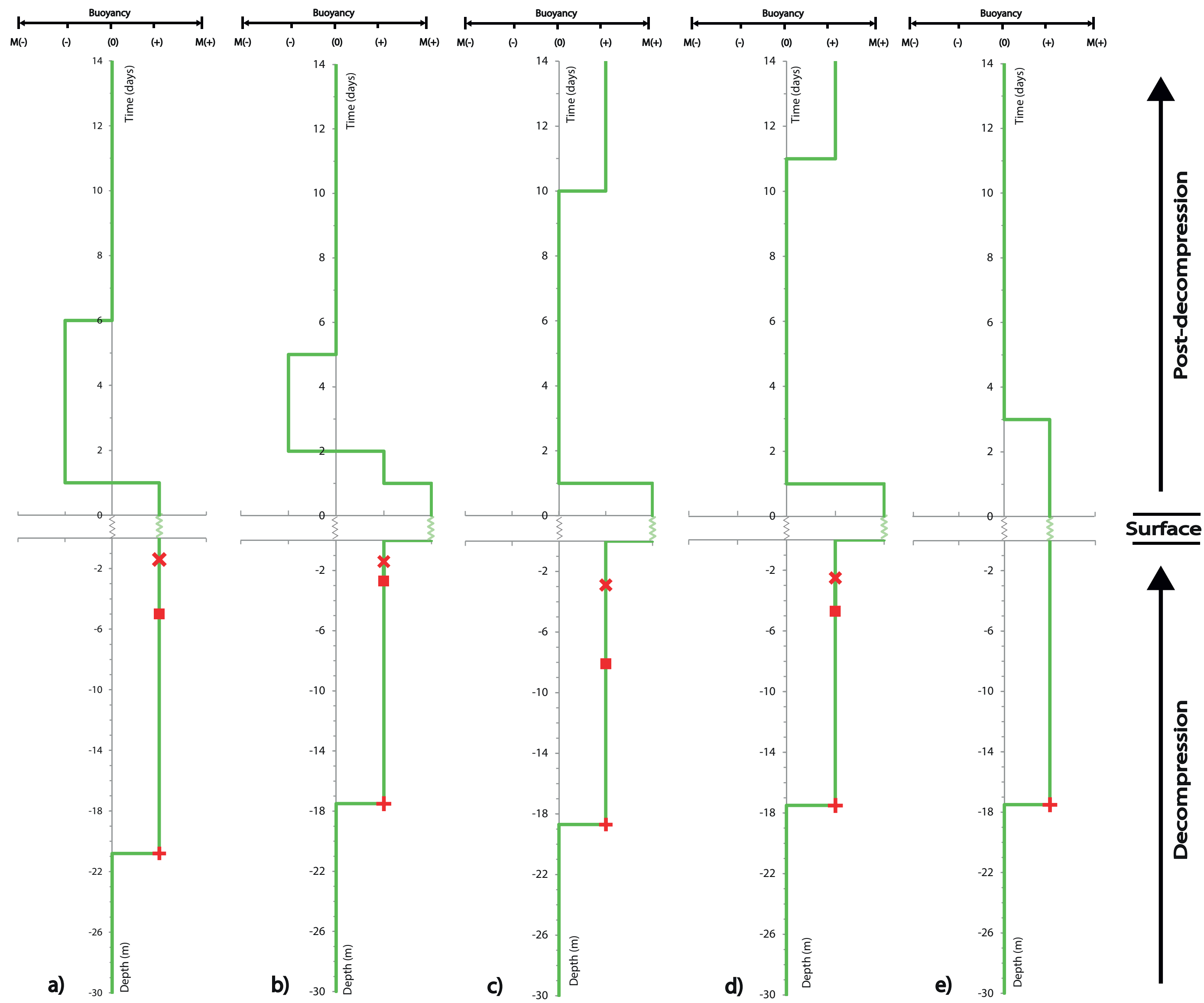

Fig. 5. Phenomena occurring during decompression and post-decompression trials of the *A. anthias* control group (n = 5, a-e). The *x-axis* indicate the buoyancy of fish (Moribund negative – M(−); negative - (−); neutral - (0); positive - (+); Moribund positive - M(+)). The *y-axis* show the decompression ascendance (negative values), and post-decompression (positive values); Red cross (+) indicate change from neutral to positive buoyancy; Red square (■) Intestinal protrusion; Red X (✖) swimbladder rupture. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

adjust to a new pressure in an ascension, will be lesser in deeper than in shallower water. Thus, as described by Jones and Scholes (1985) and Korsøen et al. (2010) for *G. morhua,* the resorption of gases in the swimbladder near the surface is lower compared to deeper waters.

During the post-decompression trial, three fish - despite being neutrally buoyant for a period of time - regained their positive buoyancy. This unusual behavior could be due to internal injuries, induced during the decompression phase that could have affected the fish. Internal injuries caused by decompression may not be visible with external inspection. However, they may affect long-term fish health. The expansion of gases in the swimbladder can lead to compression injuries of internal vital organs and emboli can cause internal hemorrhaging that may affect long-term fitness (Gitschlag and Renaud, 1994; Rummer and Bennett, 2005; Parker et al., 2006; Butcher et al., 2012). Goolish (1992) showed that killifish, *Fundulus heteroclitus*, subjected to artificial lift required a minimum of 7–8 days to restore their neutral buoyancy, in order to completely reabsorb gases in the swimbladder. This author also observed high variability amongst individuals, with some fish being unable to decrease swimbladder volume below 60–70% of its normal state. Similarly to the killifish, *A. anthias* regained neutral buoyancy after 8 days in the 25% - 12 h (in two fish), and after 13 days in the 15%-12 h (in one fish).

Assessing the effects of decompression on *A. anthias* has proven to be valuable. The average pressure reduction rate at which fish became positively buoyant was 29% pressure reduction from the initial depth of 30 m, which could indicate an approximate value of the free vertical range of this species in ascending situations. This value was similar to the 25% pressure reduction observed by Jones and Scholes (1985) while following individual cod in the sea, suggesting this was the safe limit for cod to maintain its buoyancy. Moreover, the free vertical range should correspond to < 50% pressure reduction, for cod, in natural habitats, and < 25% in pressure tank experiments. Intestinal protrusion occurs at a depth slightly below and the swimbladder ruptures at 63 and 70% of pressure reduction, respectively. Interestingly Jones and Scholes (1985) suggested similar values for wild cod at which, pressure reductions above 60% can cause swimbladder rupture.

There were two unexpected results in the control group that deserve scrutiny. Two individuals - after 24 and 48 h - went from positive to

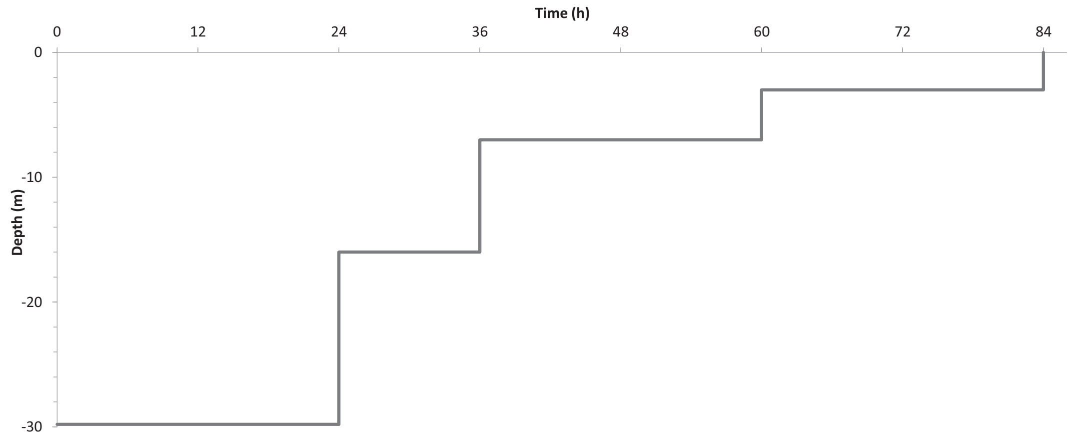

Fig. 6. Suggested decompression profile for *A. anthias*, consisting of 35% x 12 h in the first step (30 to 16 m); 35% x 24 h in the second step (16 to 7 m); 25% x 24 h in the two subsequent steps (7 to 3 m; 3 m to surface).

negative buoyancy in the post-decompression trial (Fig. 5 a and b). In both cases there was occurrence of swimbladder rupture during the ascendance, which was observed by the release of gas bubbles from the anus. Despite this fact, both individuals arrived to the surface positively buoyant (one individual was positive buoyant and the second was moribund positive). Moreover, as described above, after 48 h both fish were negatively buoyant contrarily to what was expected. We therefore hypothesized the following: during the ascendance, with the sudden decompression, the gases in the swim bladder expand, therefore compressing internal organs (Rummer and Bennett, 2005); such compression can provoke intestinal protrusion, at which point the intestine (or a small portion of the intestine) is pushed out of the abdominal cavity. If the ascension is too fast for the fish to reabsorb the excess of gas in the swim bladder, the gas overexpands, leading to swimbladder rupture (Jones and Scholes, 1985; Rummer and Bennett, 2005; Colotelo et al., 2012). As the anus of the fish is obstructed with the intestine, the gas released from the swimbladder gets trapped in the abdominal cavity. The release of small gas bubbles from the anus, observed during the ascendance, may indicate that there should be a lag between the depth recorded for swimbladder rupture, and the real depth at which this event occurred. Moreover, the observed phenomena of intestinal protrusion can be a consequence of swimbladder rupture, where the air trapped in the abdominal cavity forces the intestine evert from the anus. Consequently, the air is forced to escape from the abdominal cavity through micro-fissures in the everted intestine. We could therefore assume that swimbladder rupture could have occurred between 63 and 70% of pressure reduction. The air of the abdominal cavity escaped completely after 24 and 48 h respectively, at which point fish became negatively buoyant in the tank due to previous swimbladder rupture.

In two other fish, the same phenomena could have occurred, with a slight difference: after 24 h post-decompression, the two positively buoyant fish regained their neutral buoyancy (Fig. 5 c and d). Since fish have the ability to heal damaged swimbladder rapidly (Burns and Restrepo, 2002; Brown et al., 2010), it is possible that time needed to completely relieve the trapped gas from the abdominal cavity (approximately 24 h) was the time to heal the swimbladder, and consequently these two fish became neutrally buoyant. Burns and Restrepo (2002) found that red grouper, *Epinephelus morio*, and red snapper, *Lutjanus campechanus*, are able to heal the swimbladder sufficiently to be functional in four days. Shasteen and Sheehan (1997) reported that largemouth bass, *Micropterus salmoides*, are capable of healing their swimbladders within 17 h from a 0.5 cm hole. The fact that these two individuals required ten and eleven days to regain their positive buoyancy suggests the intensity of the rupture. Nichol and Chilton (2006) showed that ruptured swimbladders in Pacific cod when sufficiently small, the healing process is very fast but not entirely deflating the swimbladder.

In order to maintain healthy *A. anthias* individuals at the surface, for public aquarium exhibiting purposes, the framework of this study was to define a protocol for mitigating surface mortality while increasing healthy individuals in the long term. Our study suggests the need for a balance between the rate of pressure reduction and total amount of time required for surfacing the animals. Trapping the fish for a long period can induce stress behavior by spatially restricting fish movement and potentially affecting recovery (Hannah et al., 2012), as stress leads to an ionic/osmotic disturbance that can limit gas transport in blood (McDonald and Milligan, 1997). The lower pressure changes tested (15% and 25%) with longer acclimation duration (24 h) required the fish to spend 9 and 6 days in the decompression containers, respectively, while in the 35% x 24 h and 45% x 24 h the amount of days in the decompression containers were 4 and 3 days, respectively. Our work suggests that decompression profiles should not be longer than 4 days in total.

5. Conclusions

The results of this experiment were not completely clear, as far as selecting an optimum decompression profile. However, it seems plausible to combine two decompression profiles used in this experiment, at which proportion of neutrally buoyant fish were greater, both deeper and shallower steps, and the required surfacing time remained within safe limits. This profile has a total duration of 84 h and comprises 4 lifting steps with 35% reduction in the first step with 12 h acclimation, changing to 35% x 24 h in the second step. An even more conservative profile in shallower water, changing to 25% reduction in the two subsequent steps, with acclimation duration of 24 h (Fig. 6), is also advisable. It should be noted that the suggested decompression profile was built based limited information and was not tested during this study, therefore, it should be applied with caution. In addition, the protocol developed to mitigate surface mortality was designed for conditions where oceanic cages or containers can be lifted gradually. Also, decompression of the animals should be done in dark containers to reduce possible stress. Future work on the effect of different decompression profiles on barotrauma and behavior responses to pressure changes in *A. anthias.* Should analyze the effect of fish density in traps, fish size, and water temperature on fish condition.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The experiments comply with the current laws of the country in which they were performed. All applicable national and international guidelines for the handling, collecting and care of animals were followed. All fish were caught and handled through noninvasive methods. The species collected is not protected throughout its range.

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