

Effect of shelter acclimation on the post-release movement and putative predation mortality of hatchery-reared black-spot tuskfish *Choerodon schoenleinii*, determined by acoustic telemetry

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Abstract In this study, the effect of shelter acclimation on the post-release movement and putative predation mortality of hatchery-reared black-spot tuskfish *Choerodon schoenleinii* was examined using acoustic telemetry. We acclimated four one-year-old fish to shelters in cages before release, and monitored their movements with six non-acclimated fish. Since it was not possible to compare the behavioral pattern between the former and the latter fish due to the short monitoring periods of the latter fish, we also compared their movements with those of large non-acclimated fish that were less likely to be preyed upon. While 67% of the non-acclimated fish showed untypical movements before cease of the detections that suggest the predation event would have occurred, none of the acclimated and large non-acclimated fish showed the untypical movements. In addition, the probability of the cease of the detections was about 13 times lower in the acclimated than non-acclimated fish. The signal detection patterns suggest that the acclimated fish utilized night-time shelters from the first night after release while the large non-acclimated fish started to utilize shelters several days after release. Therefore, it is likely that the shelter acclimation enhanced the shelter utilization by tuskfish, possibly decreasing the post-release predation mortality.

Keywords Biotelemetry • Captive-bred • Cox proportional hazards model • Learning • Restocking • Stock enhancement • Survival analysis

Introduction

Hatchery-reared fish have been stocked into natural environments in an attempt to restore or maintain the wild populations [1–3]. One of the major pitfalls of the stocking projects is the dramatic levels of predation mortality soon after release [4, 5]. The key to better survival of released fish is to release the fish of appropriate size in the area and season where the wild juveniles are distributed [4, 6]. However, even the hatchery-reared fish of relevant size, released in the area in which the wild juveniles reside, sometimes suffer from the high level of predation mortality. This is at least partially attributed to the inability of utilization of wild habitats because the hatchery-reared fish are usually reared in a simple tank without any wild habitats.

One possible method to increase the ability of habit utilization and thus decreasing post-release predation mortality is to acclimate the fish to wild habitats before release. Both field and laboratory experiments on flounder species suggest that pre-release habitat acclimation benefits hatchery-reared fish by allowing them to adjust to the wild habitat, thus decreasing post-release predation mortality [7–10]. However, except for these studies on flounder species, only a few studies have investigated the effects of habitat acclimation on the post-release predation mortality of fish [11].

The black-spot tuskfish *Choerodon schoenleinii* is a highly prized commercial fish which has been targeted for stock enhancement in Okinawa Prefecture, Japan [12]. It lives around coral reefs from the Ryukyu Islands to the West Pacific (Fairclough DV and Nakazono A, 2004, IUCN red list of threatened species: <http://www.iucnredlist.org> “Accessed 2 February 2010”). The ecological knowledge of the tuskfish in Okinawa Prefecture has been accumulated. This species shows a clear diurnal movement pattern; it feeds on benthic prey during the day and rests in shelters at night [13–15]. The fish settles around seagrass beds after the pelagic larval periods of about a month [16] and stayed there during the summer [17, 18]. Thereafter, the fish gradually moves out of the seagrass beds to the sandy gravel areas as the fish grows [17, 18]. The pattern of the substrate preference changes as the fish grows. The fish of 10–20 mm total length (TL) prefers soft substrates such as seagrass to the hard substrates such as dead corals; the fish of 30–40 mm TL prefers hard substrates to the soft substrates [19]. The fish at least over 90 mm TL utilizes a burrow-like shelter which it excavates at the base of hard substrates, such as limestone reefs [20, 21].

Around the coast of Ishigaki Island in Okinawa Prefecture, hatchery-reared fish of about 60 mm and 100 mm TL were experimentally released onto dead coral patches which can be used by fish for sheltering within the seagrass beds based on the ecology and behavior of the wild juveniles; however, all released fish (both about 60 mm and 100 mm TL groups) disappeared from the release site within 2 weeks (Okuzawa et al., unpubl. data, 2009). Some released fish were found in the stomachs of piscivores (Okuzawa et al., unpubl. data, 2009) and interval camera deployed at the release site recorded different kinds of large piscivores (i.e. serranid, lutjanid and carangid species) gathering around the release site (Kawabata et al., unpubl. data, 2009). Post-release predation mortality is a plausible cause for this rapid disappearance; therefore, developing release strategies that reduce post-release predation mortality is a priority for ensuring the success of the stock enhancement of this species.

In the previous study, Kawabata et al. [20] conducted a laboratory predation experiment in an attempt to understand the effect of shelter acclimation on the post-release predation mortality of hatchery-reared black-spot tuskfish. We caged the tuskfish with shelters before their exposure to predatory groupers, and found that the acclimated fish utilized shelters more frequently than did non-acclimated fish and the predation rate of acclimated fish was lower than that of non-acclimated fish. Based on this knowledge, we made an assumption that keeping the tuskfish with appropriate shelters encaged and protected from predators in the release area for a period before release would enhance the shelter utilization by tuskfish and decrease the post-release predation mortality in the field.

With the advent of acoustic telemetry using automated monitoring receivers and coded acoustic transmitters, the movements of target species can be continuously monitored without active tracking [14, 15, 22–25]. After the target species implanted with a transmitter is preyed upon, the transmitter is retained in the predator's gut for a certain period [22]. Thus, this method would be applicable to identify a predation event from the signal detection pattern because the detection pattern is usually different from that of target species when the transmitter is inside the predator's gut [22]. As automated monitoring receivers require unobstructed lines of sight to record acoustically tagged fish, this method can also be used to determine if target fish associate with shelters or not [23–25].

The objective of this study was to investigate the effect of shelter acclimation on the post-release movement and putative predation mortality of the hatchery-reared black-spot tuskfish in the field. To achieve the objective, we acclimated the fish to shelters in a cage before release, and monitored

their movements with non-acclimated fish, using acoustic telemetry. Since it was not possible to compare the behavioral pattern between the acclimated and non-acclimated fish due to the short monitoring periods of non-acclimated fish, we also compared their movements with those of large non-acclimated tuskfish that were less likely to be preyed upon, monitored in 2006 and 2007 [14].

Materials and methods

Study site

This study was conducted in Urasoko Bay of Ishigaki Island, Japan (Fig. 1). The study site contains patches of corals along a deep sandy bottom (approximately >17 m in depth) surrounded by coral reefs (approximately <17 m in depth).

Sample fish and tagging

Ten one-year-old (128 ± 11 mm TL) and nine two-year-old (261 ± 30 mm TL) hatchery-reared tuskfish were monitored in 2009 and in 2006, respectively. Four of the ten one-year-old tuskfish were used as an acclimated fish group (AC fish); six of the ten one-year-old tuskfish were used as a non-acclimated fish group (NAC fish); all nine two-year-old fish were used as a large non-acclimated fish group (LNAC fish). One-year-old tuskfish were larger than the tuskfish considered to be used in an actual stocking (10–100 mm TL) because the smallest fish size that could be successfully implanted with the smallest acoustic transmitters was around 120 mm TL when the study was conducted (See Vemco Ltd. home page: www.vemco.com). The tuskfish had been reared at Yaeyama Station of the Seikai National Fisheries Research Institute, Fisheries Research Agency. Two types of coded ultrasonic transmitters that transmit a set of signals (V7-2L and V9P-2H, Vemco Ltd., Halifax, Nova Scotia, Canada, V7-2L: diameter 7 mm, length 20 mm, weight in air 1.4 g; frequency 69.0 kHz, power output 136 dB, signal interval 180–300 s, expected battery life 281 days, V9P-2H: diameter 9 mm, length 46 mm, weight in air 6.2 g; frequency 69.0 kHz, power output 147 dB, signal interval 60–180 s, expected battery life 150 days) were used in the study. V7-2L transmitters were inserted into AC and NAC tuskfish, and the V9-2H transmitters were inserted into LNAC tuskfish.

One transmitter was surgically implanted into the abdominal cavity of each fish under anesthesia using 0.1% 2-phenoxyethanol, in accordance with the Japan Ethological Society guidelines for the experimental use of animals (see Kawabata et al. [14] for details). After surgery, fish were placed in a black circular 1000-l polyethylene tank for 3–8 days and monitored for postsurgical effects. Surgery had

no observable effects on fish swimming and feeding behavior. A preliminary experiment using dummy transmitters also suggested that transmitter implant did not negatively affect the growth and survival of black-spot tuskfish [26].

Release protocols and monitoring system

To investigate the effects of shelter acclimation, AC fish were acclimated to shelters at the release site before release. The NAC and LNAC fish were directly released at the ocean bottom. For AC fish, a cubic cage without a bottom (0.6 m in length \times 0.6 m in width \times 0.3 m in height) constructed of 10-mm plastic mesh was set on the sea bottom at the release site for each fish. One tunnel-shaped, brick shelter (inside space of 40 mm in height, 80 mm in width, and 230 mm in length) for the tuskfish was deployed inside the cage. One tuskfish was introduced into each cage at between 14:00 and 16:00 on 19 August 2009 and acclimated for 5 days before release. No food was provided during acclimation. After acclimation, the AC fish were released at between 14:00 and 15:00 on 24 August 2009, along with the six NAC fish. Shelters were left at the release site. Nine LNAC fish were released on 14:05 on 22 September 2006 without acclimation. All fish were released around the central area (Station C, E and H in Fig. 1b) because there were more patches of corals which could be used by fish for sheltering than surrounding area (Station A, B, D, F, G, I and J). After the release, the signals from the implanted fish were monitored by nine or six automated monitoring receivers (VR2 and VR2W; Vemco Ltd.) that recorded the ID number, date and time for each fish (Fig. 1b, c). The transmitter detection ranges were approximately 100 m and 50 m in V9P-2H and V7-2L transmitters, respectively (Fig. 1b, c). Data were downloaded between 23 and 27 April 2007, and on 30 October 2009.

Data analyses

Data for the LNAC fish were used as the control group with low predation probability, and the detection pattern was compared to those of AC and NAC fish groups.

Survival analysis

To determine whether acclimation affected the probability of the cease of the detection (considered to be related to mortality), the Cox proportional hazards model, a widely used semiparametric survival model [27], was applied to the tuskfish monitoring data (See Kawabata et al. [28] for details). Survival models such as the Cox proportional hazards model can include individuals that never undergo the event of interest as sources of censoring data. Censoring allows the calculation of probability functions without attributing event times. The Cox proportional hazards model relates the hazard rate (instantaneous rate of event occurrence) to explanatory variables. The equation used was $h(t) = h_0(t) \exp [\sum \alpha_i X_i]$, where $h(t)$ is the hazard rate, $h_0(t)$ is the baseline hazard function, and X_i is the i th explanatory variable. The equations for the hazard ratio that compared two variables of the i th variables were $[h(t, X^*)][h(t, X)]^{-1} = \exp [\alpha_i(X^* - X)]$, where one group of probability of event occurrence corresponded to X^* and another group of probability of event occurrence corresponded to X . A hazard ratio of 1 means that there is no difference between two groups. A hazard ratio of 10, on the other hand, indicates one group has ten times the probability of event occurrence of the other group. Similarly, a hazard ratio of 1/10 implies that the one group has one-tenth the probability of event occurrence of the other group. In this study, the time detection ceased or started to be recorded exclusively at some receivers without any rhythm and movement was regarded as “the cease of the detection,” which was the event of the objective variable. The end of the monitoring due to the battery expiration or recovery of the receivers was regarded as censoring of the objective variable.

To investigate differences in the probability of the cease of the detection between AC and NAC fish groups, the fish only released in 2009 were initially examined. Both acclimation treatment and TL of the fish were evaluated as explanatory variables for estimating time to cease of the detections; the TL was included because size at release has been reported to significantly affect post-release survival [5, 29, 30] and there were some TL variations in the tuskfish released in 2009 (Table 1). The significances of these explanatory variables were then assessed by progressively removing them from the model and comparing the change in deviance using likelihood ratio test with χ^2 distribution (LR-test) [27]. The final model for estimating time to cease of the detection was also determined by progressively removing the explanatory variables when the variables were not significant in the LR-test.

To determine how much the probabilities of the cease of the detection of AC and NAC fish groups (released in 2009) differ from LNAC fish with less predation probability, both fish released in 2009 and 2006 were included in the model. Treatment (AC, NAC and LNAC) was included as an explanatory variable for estimating time to cease of the detection. TL of the fish was not included in the model this time because there was no significant effect of the size in the former analysis (see Results) and treatment represented the TL of the fish. The significance of the treatment effect was then assessed by removing it from the model and comparing the change in deviance, using LR-test. The final model for estimating time to cease of the detection was also determined by the result of the LR-test. Survival analysis was performed using R 2.8.0 (The R Foundation for Statistical Computing, Vienna, Austria) with the R library, “survival.”

Detection patterns before cease of the detections

After the target species implanted with a transmitter is preyed upon by a predator, the transmitter is retained in the predator’s gut for a certain period [22]. Thus, it would be possible to detect a predation event from the detection pattern because the pattern would be different from the target species when the transmitter is inside the predator’s gut [22]. In this study, in order to estimate whether the cease of the detection was related to predation mortality, we examined the signal detection pattern before the cease of the detection in each fish. We categorized the detection patterns into several types and compared the compositions of types among the treatments (AC, NAC and LNAC) using Fisher’s exact probability test. Fisher’s exact test was performed using R 2.8.0 (The R Foundation for Statistical Computing, Vienna, Austria) with R function “fisher.test.”

Since untypical night-time movements were found before the cease of the detections in some fish, we calculated the cumulative travelled distance the fish moved during the night in the following manner. First, for removing the effect of the overlaps of receivers’ detection ranges, the main receiver of the particular hour, $R(t)$, was defined as a receiver detecting each fish most frequently during that hour. Second, the distance between the main receiver at particular hour, $R(t)$, and the next main receiver, $R(t+1)$, was calculated. Third, the cumulative traveled distance of each fish during the night (20:00–6:00) was calculated by summing these distances as explained above. The maximum value of the cumulative

traveled distance of each fish was then determined and tested if there was any extreme value, over 1.5 times of the upper quartile, from those of the other fish. We progressively removed the extreme values until there were no extreme values. We also fitted non-extreme values into truncated normal distribution due to a lot of zero values, and calculated the probability of the each extreme value. These statistical analyses were performed using R 2.8.0 (The R Foundation for Statistical Computing, Vienna, Austria) with R function “boxplot,” “truncnorm” and “fitdistr (MASS).”

Post-release shelter utilization patterns

When the fish does not leave the monitoring area, the detection period can be used as an index of how long the fish associates with shelters because the automated monitoring receivers require unobstructed lines of sight to detect the fish [28–30]. To determine the post-release shelter utilization pattern of each treatment group, the detection period obtained during the first 10 days after release were analyzed. A 10-day period was used because the LNAC fish started utilizing shelters within 10 days after release (Kawabata et al., unpubl. data, 2009). The detection periods were calculated as follows. First, because each transmitter does not pulse regularly, each fish was assumed to be out of the shelters during any 10-min period when the fish was detected by at least one receiver. Second, the number of daily detection periods during the day (8:00–18:00) and at night (20:00–6:00) was counted for each fish. Daily detection periods were analyzed by defining daytime as 8:00–18:00 and night-time as 20:00–6:00. Data obtained during 6:00–8:00 and 18:00–20:00 were not used in this study, thus avoiding fluctuations in sunrise/sunset times and crepuscular periods of the study area. Since the detection period was also affected by each fish’s horizontal movements (i.e., movements in and out of the receivers’ detection range), any fish showing movements away from the monitoring area was eliminated from the analyses. That is, a fish that was detected more than 50% of the time by the outside receivers (A, B, D, F, G, I and J) was eliminated because that fish was likely to move out of the detection range of the receivers.

A general linear model (LM) was used to determine whether the daily detection period changed over time and there was any difference among treatments. The daily detection period was regarded as the objective variable, while the time, treatment and their interaction were regarded as explanatory variables. The detection periods during the day and at night were analyzed separately. The statistical significances of

these explanatory variables were assessed using the values of F . A general linear model analysis was performed using R 2.8.0 (The R Foundation for Statistical Computing, Vienna, Austria) with R function “lm.”

Long-term diel detection patterns

The long term diel detection patterns were also analyzed to provide an insight on daily movements of the individuals monitored for long periods. As mentioned above, the fish was assumed to be out of the shelters (1) or in shelters (0) during any 10-min period. The time-series shelter utilization data (1 or 0) was then subjected to fast Fourier transform (FFT) to understand the long-term cyclical shelter utilization pattern of the fish. The FFT was performed using Igor Pro 6.0 software (WaveMetrics, Lake Oswego, OR, USA). Since most fish showed strong diel patterns, the detection periods during the day and at night were compared using t -test.

Results

General results

The summary of the monitoring data for hatchery-reared black-spot tuskfish is presented in Table 1. Most of the fish were continuously detected by at least one receiver per day throughout the period that the fish were within the monitoring area (Table 1).

The signals of one of the four AC fish (AC2) and four of the nine LNAC fish (LNAC1–3, 8) had been recorded until the end of the monitoring (either receivers' recovery or over the expected battery life); therefore, these fish were considered as the sources of censored data in the survival analysis and were not used for the analysis of the “detection patterns before cease of the detection” (Table 1). The signals of the other three of the four AC fish (AC1, 3, 4), all NAC fish (NAC1–6) and five of the nine LNAC fish (LNAC4–7, 9) were either ceased or started to be recorded exclusively at some receivers without any rhythm and movement 1–104 days after release; thus, these fish were considered as the event data or “cease of the detection” in the survival analysis and were analyzed in the “detection patterns before cease of the detection.”

The signals of two of the four AC fish (AC1, 2) and eight of the nine LNAC fish (LNAC1–8) were recorded for over 10 days after release. One fish (LNAC6) was only detected intermittently at Station B and second fish (LNAC8) was mainly detected at Station B (67% of the time), indicating that these fish may move into and out of the monitoring area. Therefore, these two fish LNAC6 and 8 were not used for the analysis of the “post-release shelter utilization patterns” and “long-term diel detection patterns” even though the total monitoring periods exceed 10 days.

Survival analysis

When AC (AC1–4) and NAC fish (NAC1–6) were examined, there was a significant effect of the treatment on the probability of cease of the detection (Fig. 2; LR-test, $X^2 = 9.63$, $df = 1$, $P < 0.01$); while there was no significant effect of size on the probability of cease of the detection (LR-test, $X^2 = 2.24$, $df = 1$, $P = 0.13$). The model composed of the effects of the treatments was chosen as the final model based on

the results of the LR-test (Table 2). The hazard ratio (exponential of the coefficient) in the model was approximately 13 (Table 2), indicating that the signal of NAC fish group was about 13 times more likely to cease than that of AC fish group.

When AC (AC1–4), NAC (NAC1–6) and LNAC fish (LNAC1–9) were examined, there was a significant effect of the treatment on the probability of cease of the detection (Fig. 2; LR-test, $\chi^2 = 14.3$, $df = 2$, $P < 0.01$). The model composed of the effect of the treatment was chosen as the final model based on the results of the LR-test (Table 2). The hazard ratio between AC and LNAC fish was approximately 3, while the ratio between NAC and LNAC fish was approximately 28 (Table 2). The results indicate that the signals of AC and NAC fish groups were about 3 and 28 times more likely to cease than that of LNAC fish group, respectively.

Detection patterns before cease of the detections

Only the fish whose detection ceased during the monitoring period were analyzed in this section. Three distinctive detection patterns (Type A–C) have been found before cease of the detections in the monitored tuskfish (Fig. 3; Table 3). Type A: the signals of two of the three AC (AC1 and 4; 66.7%), one of the six NAC (NAC1; 16.7 %) and five of the five LNAC fish (LNAC4–7 and 9; 100%) showed diurnal movements in the restricted ranges (1–4 receivers) which are typical to the black-spot tuskfish [14, 15], before the cease of the detection (Fig. 3a). Type-B: the signals of four of the six NAC fish (NAC2, 3, 5, 6; 66.7%) showed active movements with wide movement ranges (7–10 receivers) both during the night and at night, which are untypical to the tuskfish before the cease of the detections (Fig. 3b). The maximum cumulative traveled distances during the night of the four fish were categorized into extreme values and their probabilities were less than 0.01 % in the fitted truncated normal distribution (Fig. 4). The signals of two of the four fish started to be recorded exclusively at some receivers without any rhythm and movement after the untypical movements (Fig. 3b). Type C: very small numbers of signals were recorded in one of the three AC (AC3; 33.3%) and one of the six NAC fish (NAC4; 16.7%) (Fig. 3c). There was a significant difference in composition of the detection types among treatments (Table 3; Fisher's exact probability test, $df = 4$, $P < 0.05$).

Post-release shelter utilization patterns

Only the fish that were continuously monitored for over 10 days after release were analyzed in this section (AC1, 2, LNAC1–5 and 7). In LNAC fish, there were detections both during the day and at night for the first few days after release and the detection periods both during the day and at night decreased over time (Figs. 5a, 6). On the other hand, in AC fish, there were almost no detections at night from the first night after release, even though the detection periods during the day decreased over time as LNAC fish (Figs. 5b, 6). In the analysis of the detection periods during the day, the effect of time was significant ($F = 29$, $df = 1$, $P < 0.01$) but the effect of treatment and the interaction were not significant ($F = 2.43$, 1.70 , $df = 1, 1$, $P = 0.12, 0.20$, respectively) (Table 4). In the analysis of the detection periods at night, the effects of time, treatment and the interaction were significant ($F = 17.7, 7.96, 6.30$, $df = \text{all } 1$, $P < 0.01, <0.01, <0.05$, respectively) (Table 4).

Long-term diel detection patterns

In the long-term diel detection pattern analysis, the fish showed clear diurnal movement patterns with restricted ranges. The signals were recorded more frequently during the day and less frequently at night in all of the eight tuskfish examined (t -test, $P < 0.01$). FFT revealed dominant 24.4 h peak in detections for all of the 8 tuskfish examined. The signals were mainly recorded by 1–4 receivers (Station A, B, C and E).

Discussion

In this study, we attempted to identify a predation event from the signal detection pattern because the detection pattern would be different from that of target species when the transmitter was inside the predator's gut [22]. The black-spot tuskfish is a diurnal species with a restricted movement range [14, 15]; however, the detection Type B was apparently different from the typical diurnal restricted movement patterns of tuskfish; the signals were recorded frequently both during the day and at night with wide movement ranges (Fig. 3b, 4). The signals from the LNAC fish (that were unlikely to be preyed upon) were recorded at night for a first few days after release but were mainly recorded by only one receiver and no movements around several receivers were found (Fig. 4, 5a). Even though the differences of diel activity patterns between hatchery-reared and wild fish were reported in some species [24, 31], none of the hatchery-reared black-spot tuskfish examined both in laboratories and in fields in the previous studies [14, 15, 20, 32] showed untypical movement patterns (active movements both during the day and at night) like Type B fish. In addition, the detection patterns of two of the four Type B fish suggest that the transmitters were on the sea bottom after the untypical detection patterns (Fig. 3b). Moreover, the detection patterns of the Type B fish were similar to those of the candidate predators (i.e. serranids and lutjanids) (Kawabata et al., unpubl. data, 2008). Considering the above, the fish with Type B detection patterns were probably preyed upon by these predators soon after release, the signals from the transmitters in predators' guts were recorded for a few days, and the transmitters of some of the fish were dropped on the sea bottom in the monitoring area along with the excretion of feces from predators.

Given the estimation of the predation event mentioned above, the results of the study would suggest that the effects of shelter acclimation decrease the post-release predation mortality of the hatchery-reared black-spot tuskfish. While 67% of the NAC fish showed detection Type B that suggests the predation event would have occurred, none of the AC fish or LNAC fish showed detection Type B before cease of the detections. In addition, the probability of the cease of the detections was about one-thirteenth in the AC as much as the NAC fish. Moreover, the rate of predation mortality was lower in shelter-acclimated than non-acclimated fish in the previous laboratory predation experiment [20]. Considering these facts, the acclimation to shelters before release might decrease the post-release predation mortality of hatchery-reared black-spot tuskfish in the field, even though the further research

directly assessing the predation mortality should be conducted for confirming the effect of the shelter acclimation on the predation mortality in the field.

Then, why did the shelter acclimation might decrease the post-release predation mortality of hatchery-reared black-spot tuskfish? This is probably because the AC tuskfish could utilize shelters soon after release. The post-release signal detection patterns suggest that the AC fish had utilized night-time shelters from the first night after release while the LNAC fish started to utilize night-time shelters some days after release (Figs. 5, 6). Diurnal coral reef fishes are reportedly most vulnerable to predators at dusk, and fish which have utilized shelters poorly—or not at all—are more likely to be preyed upon by predators [33]. The LNAC fish were probably large enough to avoid predators even without shelters but the smaller NAC fish were probably preyed upon by predators during the periods without shelters. Even though it was not determined which factor (familiarization of shelters, availability of shelters, alleviation of the handling stress, or combination of these factors) enhanced the shelter utilization in this study, it is highly likely that the shelter acclimation enhanced the shelter utilization by tuskfish and possibly decreased the post-release predation mortality.

The AC and LNAC fish monitored for over 10 days showed clear diurnal activity patterns with restricted movement ranges; the detection periods were longer during the day than at night and signals were mainly detected at 1–4 receivers. The decreased detection periods suggest the fish associate with shelters at night because the receiver and transmitter need unobstructed line-of-sight to record the signals from transmitter. The wild black-spot tuskfish is a diurnal territorial fish: it moves actively during the day for feeding on benthic prey and rests in a shelter at night [13, 15]. Considering these facts, the fish monitored for long periods showed consistent diel behavioral pattern to the wild counterparts.

Even though shelter acclimation probably decreased the predation mortality rate, the probability of the cease of the detection was still approximately 3 times higher in AC fish than LNAC fish. These are several possible factors affecting the higher probability of the cease of the detection. One possibility would be that the AC fish were more likely to be preyed upon by some diurnally active predators such as carangid species than LNAC fish. The brassy trevally *Caranx papuensis*, a diurnal transient piscivore, is one of the candidates as a major predator on released tuskfish (Okuzawa et al., unpubl. data, 2009), and if a predation event by this species occurred, differentiation between active emigration and predation would be difficult because patterns of the cease of the detections would be

similar to each other. Another possibility might be intraspecific and interspecific competition. Intraspecific and interspecific competitions have been reported for many coral reef fishes [33–36], and, in general, small individuals are weak competitors compared to the large individuals [34, 35]. It is, therefore, possible that the AC fish were more likely to be forced to leave the monitoring area than LNAC fish. Since the monitoring year was different, the possibility cannot be discounted that the unspecific factors such as abundance of predators, prey and available coral patches for shelters were different between in 2006 and in 2009, causing the higher probability of cease of the detections in 2009.

Even though the size of the fish used in this study (128 ± 11 mm TL) was larger than the fish considered to be used in the actual stocking (10–100 mm TL), the method used in this study would be applicable to the size of the tuskfish used in actual stocking for the following reasons. The fish around 90–100 mm TL starts to utilize sandy/gravel areas as larger fish [17] and the fish at least 90 mm TL utilize the burrow-like shelters as larger fish [20]. Moreover, in the previous laboratory experiment, we found that the fish 90 mm TL acclimated to shelters in the similar manner to this study have lower predation mortality rate than non-acclimated fish during the exposure to the predatory groupers [20]. Therefore, the method used in this study would be applicable to the size of the tuskfish 90–100 mm TL which is within the size range considered to be used for the actual stocking.

Our study experimentally showed that pre-release shelter acclimation might decrease post-release predation mortality in the field. Further studies modified to large scale and directly assessing the predation mortality are now needed to verify the possibility of this method for the success of the stock enhancement of this species.

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Figure captions

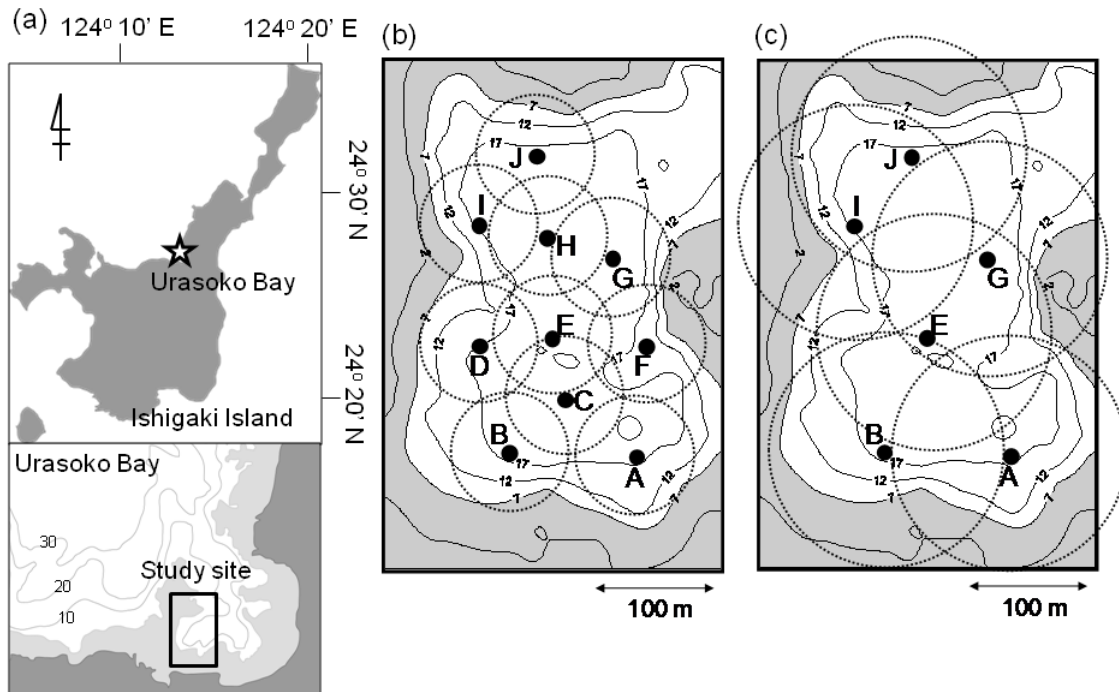


Fig. 1 Map of Ishigaki Island (a), location of Urasoko Bay (*open star*) and study site showing receiver locations (Station A–J; *solid circles*) in 2009 (b) and in 2006 (c). Lines with numbers in the map indicate the contours of the bottom depth. *Shaded* and *white areas* in the map represent areas of coral reef and sandy bottom, respectively. *Dashed circles* represent the expected detection ranges

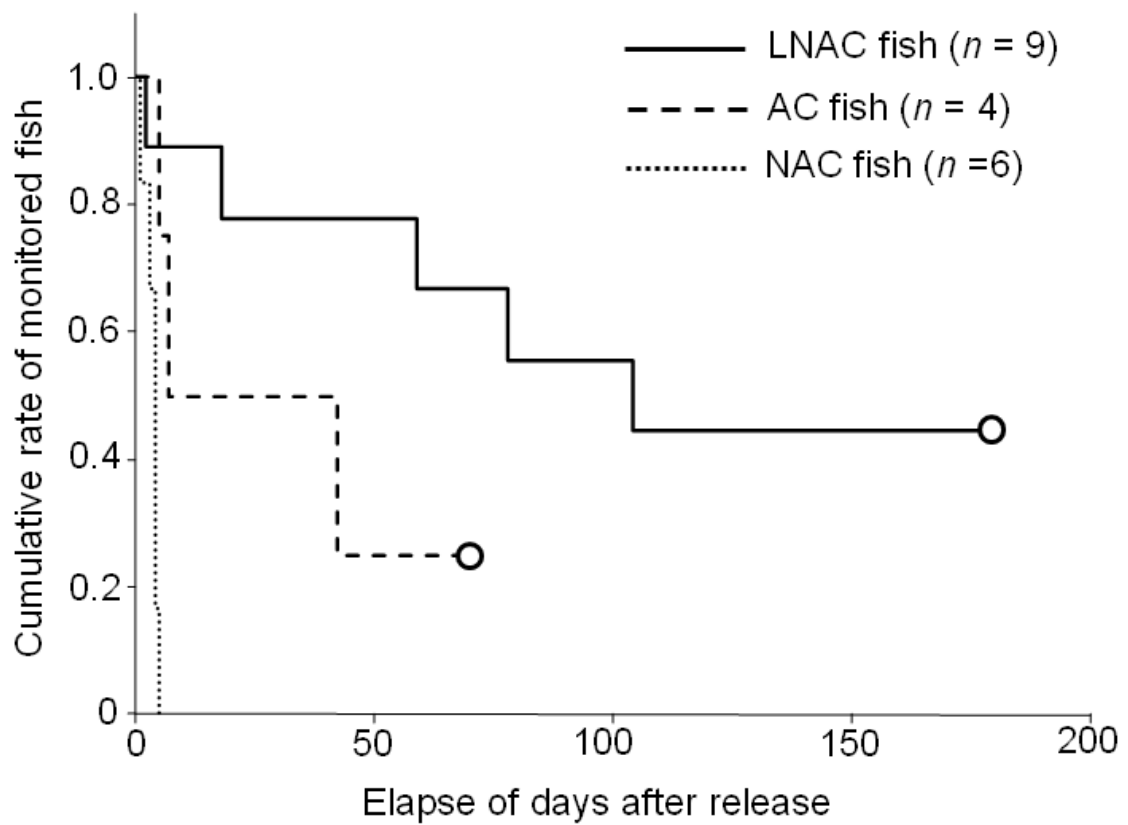


Fig. 2 Time-series cumulative rate of the monitored fish in each treatment. There was a significant treatment effect on the probability of cease of the detections (Likelihood ratio test, $\chi^2 = 14.3$, $df = 2$, $P < 0.01$). *LNAC fish* denotes large non-acclimated fish, *AC fish* denotes acclimated fish and *NAC fish* denotes non-acclimated fish. *Circles* represent the end of the monitoring due to the recovery of receivers or transmitter battery expirations

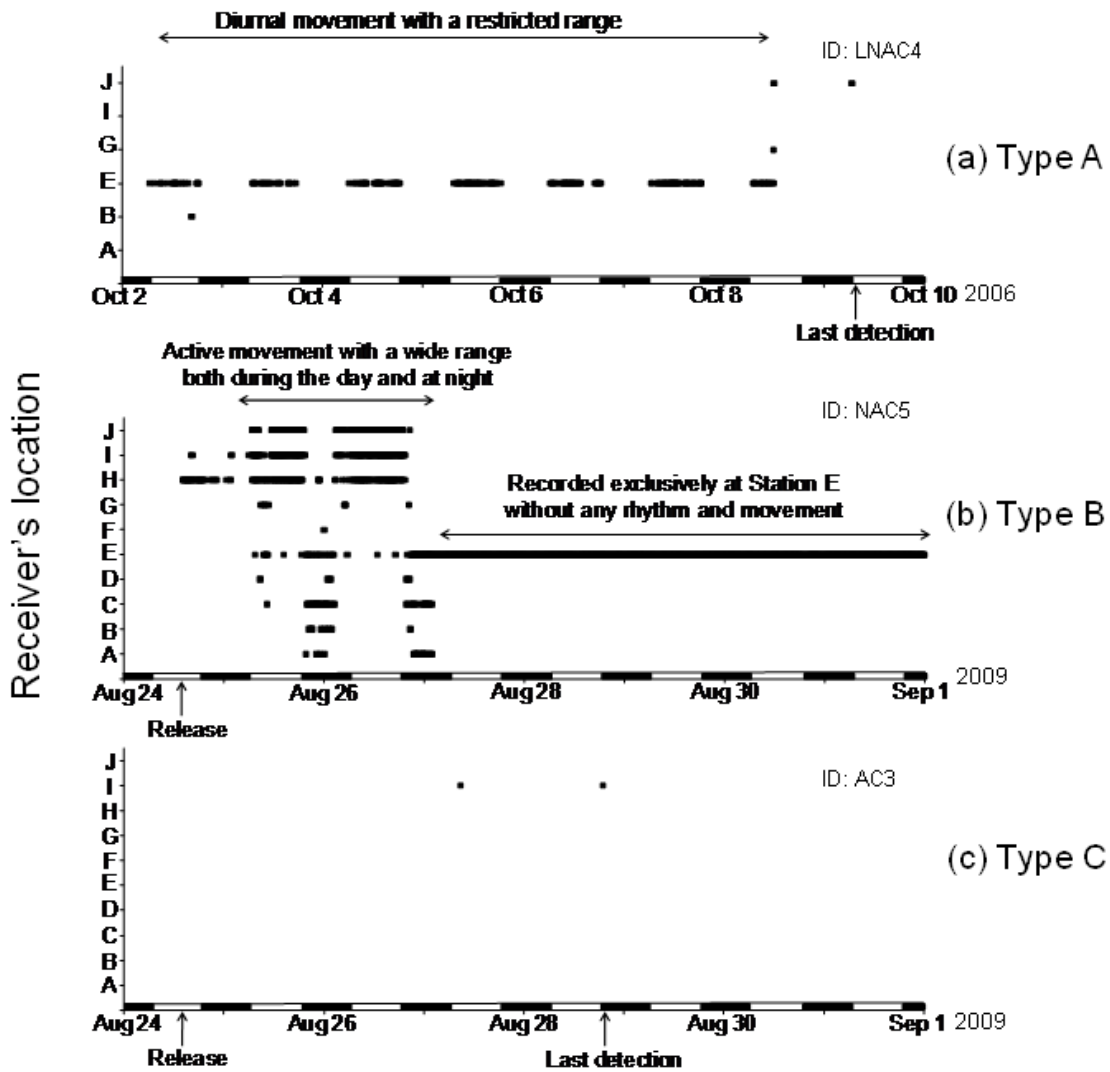


Fig. 3 Three different detection patterns before cease of the detections (Type A–C). *Black bars* at the bottom of each figure indicate night-time. The fish (e.g., LNAC4, see Table 1) with Type A showed diurnally active movements with restricted ranges. The fish (e.g., NAC5) with Type B showed untypical active movements with wide ranges both during the day and at night. Very small numbers of signals were recorded in the fish (e.g., AC3) with Type C

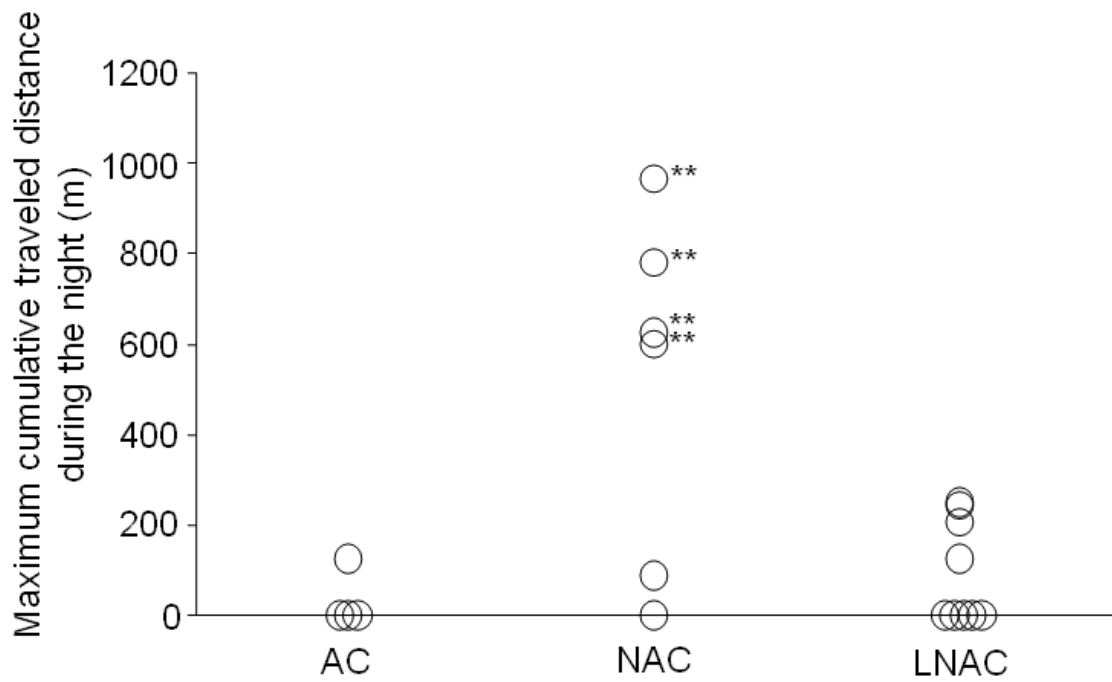


Fig. 4 Maximum cumulative traveled distance during the night in each fish. *LNAC fish* denotes large non-acclimated fish, *AC fish* denotes acclimated fish and *NAC fish* denotes non-acclimated fish.

**The values of four of the six *NAC fish* were categorized into extreme values (fitted truncated normal distribution, $P < 0.0001$)

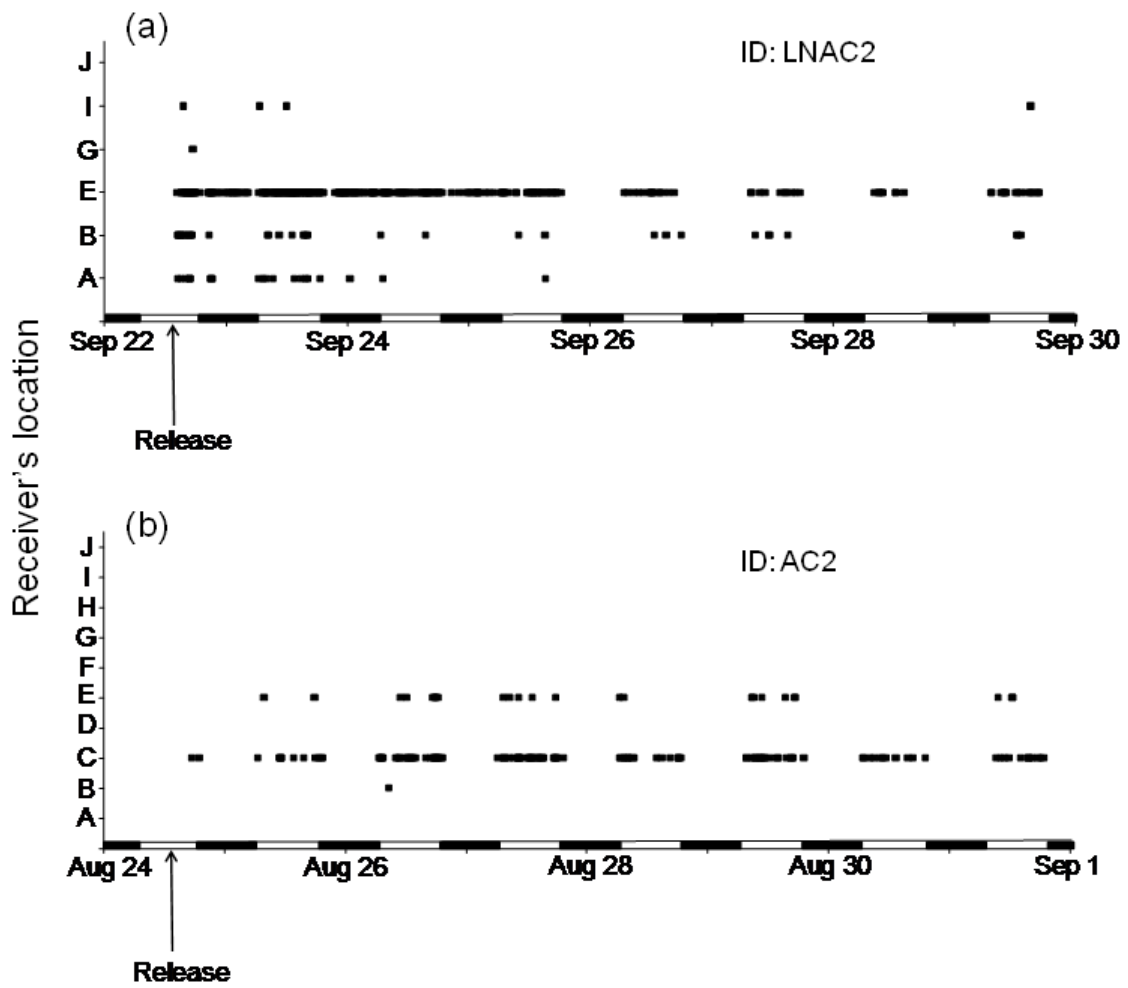


Fig. 5 Typical post-release detection patterns of large non-acclimated (LNAC) fish (a) and acclimated (AC) fish (b). *Black bars* at the bottom of each figure indicate night-time. While there were detections at night for the first three days after release in the LNAC fish, there were no detections at night from the first night after release in the AC fish

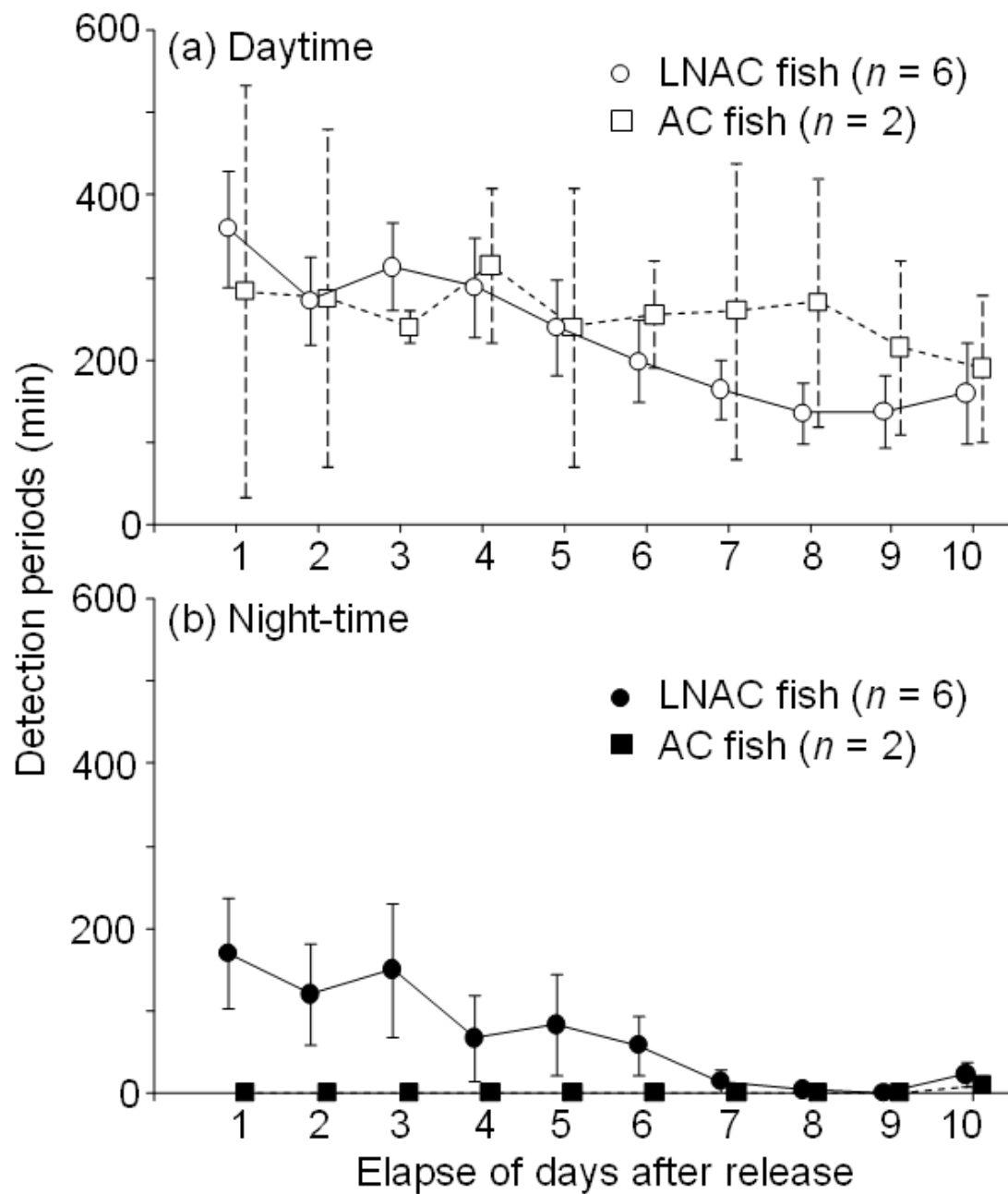


Fig. 6 Time-series daily detection periods of large non-acclimated (LNAC) and acclimated (AC) fish during the day (a) and at night (b). *Vertical lines* indicate standard error. There was a significant effect of time ($F = 29.45$, $df = 1$, $P < 0.01$) but there were no significant effects of treatment and the interaction ($F = 2.43$, 1.70 , $df = 1, 1$, $P = 0.12$, 0.20 , respectively) on the detection periods during the day. There were significant effects of time, treatment and the interaction on the detection periods at night ($F = 1.77$, 7.96 , 6.30 , $df = \text{all } 1$, $P < 0.01$, < 0.01 , < 0.05 , respectively)

Table 1 Acoustic monitoring data for 19 black-spot tuskfish *Choerodon schoenleinii*

ID	Treatment	TL (mm)	BW (g)	Transmitter type	Date released (dd/mm/yyyy)	Last detection (dd/mm/yyyy)	Overall detection periods (days)	Rate at liberty ^a (%)	Censored or detection ceased (0 or 1)	Type of cease of detection ^b (A, B or C)
AC1	AC	126	45	V7-2L	24/08/2009	04/10/2009	42	100	1	A
AC2	AC	123	40	V7-2L	24/08/2009	30/10/2009	68	100	0	-
AC3	AC	154	80	V7-2L	24/08/2009	28/08/2009	5	40	1	C
AC4	AC	137	57	V7-2L	24/08/2009	30/08/2009	7	100	1	A
NAC1	NAC	128	47	V7-2L	24/08/2009	28/08/2009	5	100	1	A
NAC2	NAC	118	38	V7-2L	24/08/2009	27/08/2009	4	100	1	B
NAC3	NAC	122	38	V7-2L	24/08/2009	27/08/2009	4	75	1	B
NAC4	NAC	126	42	V7-2L	24/08/2009	24/08/2009	1	100	1	C
NAC5	NAC	125	41	V7-2L	24/08/2009	26/08/2009	3	100	1	B
NAC6	NAC	120	38	V7-2L	24/08/2009	27/08/2009	4	100	1	B
LNAC1	LNAC	285	424	V9-2H	22/09/2006	19/03/2007	179	99	0	-
LNAC2	LNAC	300	493	V9-2H	22/09/2006	19/03/2007	179	100	0	-
LNAC3	LNAC	260	313	V9-2H	22/09/2006	19/03/2007	179	100	0	-
LNAC4	LNAC	250	334	V9-2H	22/09/2006	09/10/2006	18	100	1	A
LNAC5	LNAC	270	337	V9-2H	22/09/2006	03/01/2007	104	100	1	A
LNAC6	LNAC	280	405	V9-2H	22/09/2006	19/11/2006	59	19	1	A
LNAC7	LNAC	235	229	V9-2H	22/09/2006	08/12/2006	78	100	1	A
LNAC8	LNAC	270	368	V9-2H	22/09/2006	19/03/2007	179	100	0	-
LNAC9	LNAC	200	143	V9-2H	22/09/2006	23/09/2006	2	100	1	A

TL, total length; BW, body weight; AC, acclimated fish; NAC, non-acclimated fish; LNAC, large non-acclimated fish; Type A, fish showed diurnally active movements with restricted ranges; Type B, fish showed untypical nocturnally active movements with wide ranges; Type C, very small numbers of signals were recorded in the fish; -, there were detections until the recovery of receivers or transmitter battery expirations

^a Rate of days in monitoring area between date of release and last detection of the fish, Equation: [Rate at liberty] = [Total days monitored]/([Date of last detection] – [Date of release] + 1) * 100

Table 2 Parameter estimates for explanatory variables in each model

Model	Coefficient	SE (coeficient)	Hazard ratio ^a
<u><i>AC and NAC</i></u>			
NAC vs. AC	2.552	1.132	12.831
<u><i>AC, NAC and LNAC</i></u>			
AC vs. LNAC	1.170	0.834	3.230
NAC vs. LNAC	3.350	1.011	28.360

AC, acclimated fish; NAC, non-acclimated fish; LNAC, large non-acclimated fish

^a exponential of the coefficient

Table 3 Compositions of detection types (Type A-C; Fig. 3) before cease of the detections in each treatment

	Type A	Type B	Type C	Total
AC	2 (66.7 %)	0 (0 %)	1 (33.3 %)	3
NAC	1 (16.7 %)	4 (66.7 %)	1 (16.7 %)	6
LNAC	5 (100 %)	0 (0 %)	0 (0 %)	5

There was a significant difference in composition of the detection types among treatments (Fisher's exact probability test, $df=4$, $P < 0.05$)

AC, acclimated fish; NAC, non-acclimated fish; LNAC, large non-acclimated fish

Type A, fish showed diurnally active movements with restricted ranges; Type B, fish showed untypical active movements with wide ranges both during the day and at night; Type C, very small numbers of signals were recorded in the fish

Table 4 Effects of treatment (acclimated, non-acclimated and large non-acclimated), time and their interaction on detection periods during the day and at night, as determined by general linear model.

Detection periods during the day and at night were analyzed separately

Day	<i>df</i>	SS	MS	<i>F</i> -ratio	<i>P</i> -value
Treatment	1	390.1	390.1	2.43	0.12
Time	1	4725.4	4725.4	29.45	<0.01**
Treatment x Time	1	272.1	272.1	1.70	0.20
Residuals	76	12193.9	160.4		
Night	<i>df</i>	SS	MS	<i>F</i> -ratio	<i>P</i> -value
Treatment	1	828.8	828.8	7.96	<0.01**
Time	1	1846.7	1846.7	17.73	<0.01**
Treatment x Time	1	656.4	656.4	6.30	<0.05*
Residuals	76	7917.7	104.2		

* There was a significant effect on the detection period ($P < 0.05$). **There was a significant effect on the detection period ($P < 0.01$)