Outplanting large adult green abalone (*Haliotis fulgens*) as a strategy for population restoration

NANCY L. CARUSO*

Get Inspired, Inc., 6192 Santa Rita Ave., Garden Grove, CA 92845, USA (NLC)

*Correspondent: nancy@getinspiredinc.org

Wild abalone populations are in decline around the globe. Given their high market value, abalone have been targeted for restoration in many areas where they were once abundant. Efforts to restore California green abalone (Haliotis *fulgens*) have had limited success for species recovery. This study aimed to use large (>14cm) adult green abalone as a strategy for restoration. Abalone of this size have few predators and are generally emergent, making them more visible during surveys. Sixty-nine large (average size 16.2 cm) farm raised abalone were outplanted in three batches (May, July and August) in Newport Beach, California, on natural reef structure at a depth of 8.4 m, monitored for 15 months, and then recaptured. Using multiple tagging devices and rigorous monitoring resulted in 40% survival at the end of the study, with 61% of the mortalities occurring within the first 30 days of outplanting, and 46% of the August outplants surviving to the end of the study period. Most of the trackable abalone movements, throughout the study, were confined to a 10 m radius of outplanting areas and 79% (22) of the surviving abalone stayed within 8 m of the outplant areas.

Key words: abalone, adult abalone, *Haliotis fulgens*, outplanting, restoration, restocking, size, stock enhancement

Abalone populations worldwide have been in decline for many decades (Campbell 2000). Over fishing, illegal harvest, disease and habitat degradation are thought to be the primary causes (Cook 2014). California once supported fisheries for five species of abalone (black, green, pink, red, white) and by 1998 all commercial and recreational fisheries were closed south of San Francisco bay. Rogers-Bennett et al. (2004) found that adult abalone densities in southern California were two orders of magnitude below the estimated minimal viable population of 2000 individuals/ha and at that point, abalone recruitment in southern California had declined 20-fold over the previous decade. Despite 20 years of closed fisheries, populations of all five of these abalone species have yet to rebound on coastal reefs in southern California indicating a need for restoration activities. McCormick et al. (1994) suggested that seeding areas with hatchery raised abalone may be the only means of increasing coastal abalone stocks on a time scale meaningful to fishery managers.

The challenges facing abalone restoration include: captive spawning and rearing, protecting aggregated or outplanted animals from poaching, tracking reproduction, quantifying survival, and maximizing survival of captive-reared abalone in the wild (Henderson et al. 1988, Tegner and Butler 1989, Tegner 1992, Rogers-Bennett and Pearse 1998, Tegner 2000). Reseeding or outplanting projects have most often involved larvae and juveniles (0-100 mm) and have had mixed results around the globe with Japan and New Zealand reporting higher than 50% survival for some projects (Saito 1984, Schiel 1993, Kojima 1981). Results for reseeding or outplanting juveniles in southern California report much lower recovery rates ranging from 0-6% (Tegner and Butler 1985, McCormick et al. 1994, Davis 1995, Chick et al.2013). Quantifying recovery rates is a challenge for comparisons of efforts across time, species and different geographic areas.

Green abalone, (*Haliotis fulgens; Philippi*), are native to southern California and range from Point Conception, California, USA, to Magdalena Bay, Baja California, Mexico, and include the offshore islands (Cox 1962). They were once part of a large recreational and commercial fishery, and have previously been a target for species recovery. The green abalone is listed as a federal Species of Concern (NOAA 2004) and based on historic landings, is estimated to be at less than 1% of its baseline density (Rogers-Bennett et al. 2002). The major threat to remaining populations is their low densities and the possibility of reduced reproduction resulting from the Allee effect (Allee 1931). Low densities of broadcast spawners can lead to poor fertilization and recruitment failure because of the distances between males and females (Babcock & Keesing 1999). Remnant populations are comprised primarily of solitary abalone, many of which may not be contributing to reproduction and are thus functionally sterile (Taniguchi et al. 2013). Results from a drift tube study by Tegner and Butler (1985) indicated that in the absence of local broodstock, a fishery closure alone would not be an effective management policy for the recovery of green abalone populations on the mainland in southern California.

There have been several attempts at restoration of green abalone beginning in the 1970s. Most attempts have involved outplanting small hatchery reared animals generally due to costs associated with raising this slow growing mollusk. Seeding or outplanting results are affected by many variables including condition of the abalone at release, size, planting method, season, as well as site specific conditions including habitat type, food availability, predation, and topography (Saito 1984, Schiel 1993, McCormick et al. 1994). Because of the cryptic and mobile nature of small abalone it is difficult to estimate survival in most studies (Breen 1992, Shepherd & Breen 1992). Juveniles are highly cryptic and are found during daylight hours beneath rocks or in the recesses and crevices; they move freely at night and seldom return to the same location as the preceding day (Leighton 2000). Outplanting activities in Baja California with approximately 20 mm (shell length) green and pink abalone have yielded recovery rates ranging up to 4.7% (Sercy-Bernal et al. 2013). In summary, abalone outplanting has many variables to consider and there has been no formula for "success" that works for all species in all locations.

Translocation of abalone involves aggregating wild animals into one location with the aim of increasing reproductive success. A recent trial involving the translocation of adult California green (*H. fulgens*), and pink (*H. corrugata*), abalone showed that green abalone were not a good candidate for this restoration technique because they exhibited site infidelity (Taniguchi et al. 2013). A previous trial of 4,453 translocated green abalone on the Palos Verdes Peninsula, California was inconclusive due to poaching of the aggregated animals in the second year of the project (Tegner 1992).

Natural mortality of juvenile abalone may vary with location, time, and generally declines with age (Tegner and Butler 1985, Prince et al. 1988, Shepherd and Daume 1996). Initial mortality rates for outplanted juvenile abalone species are quite high and the rates decrease as the abalone grow to larger sizes (Schiel 1993). Saito (1984) found that survival of outplanted abalone increased with seed size in the range of 10 to 50 mm. Outplanting large adults in high densities on isolated reefs seems to be more effective (Coates et al. 2013).

Studies conducted in the 40 years before this project noted issues with the following: tagging (tags falling off, not identifiable); tracking (outplanted animals were not surveyed with enough frequency, were too cryptic, or emigrated off study site); predation (the size of the outplanted animals were vulnerable to multiple predators); poaching; and mortalities from transport shock. With historically limited success in green abalone restoration utilizing juveniles, the aim of this study was to use large (>14 cm) adult abalone for outplanting as a possible restoration strategy and to quantify their survival. This project aimed to also address some of the previous noted issues by using multiple tags, surveying with greater frequency, minimal handling in transport, and removing sea star predators. The use of large animals may act as a model for other abalone species including the endangered white (*H. sorenseni*) and black abalone (*H. cracherodii*) as recommended by Davis et al. (1998). The results are compared with previous restoration studies to determine if larger (>14 cm) outplants yield higher survival rates. The premise is that, large abalone have fewer predators and they are more easily detected and tracked.

MATERIALS AND METHODS

Study site.—The green abalone outplant site was located in Crystal Cove State Park, Orange County, California, with coordinates $33^{\circ} 34^{\circ} 6.528^{\circ}$ N, $33^{\circ} 34^{\circ} 6.528^{\circ}$ W. The study site was chosen because it was familiar to the author, too far from shore for shore divers to reach, and was not a well-known recreational dive spot minimizing opportunities for poachers. Surveys were conducted to characterize the composition of the reef, describe the topography, and assess the predator population. Predators of large abalone (>14 cm) in Orange County include octopus (*Octopus sp.*), sea stars (*Pisaster sp.*), and the bat ray (*Myliobatis californica*). The surveys were conducted using two different methods. In one method, an observer conducted two $30 \ge 2$ m band transect surveys and the other method included 30 random 1-m² quadrats along two 30-meter transects. Each surveyor collected information on reef composition (continuous reef, boulder, sand, or cobble on every meter), changes in rugosity (change in height of the reef at every meter), percent cover (sessile invertebrates, algal species), the presence of wild abalone, and presence/absence of predators.

The 450 m² reef was roughly rectangular and was divided into eight quadrants (approximately 9 x 6 m) using plastic clothesline stretched out across the reef and tied off to cinderblocks. Each quadrant was labeled with floating numbers to make the process of mapping the locations of abalone easier for volunteers. The large Pisaster stars were removed before outplanting and continuously removed during the project period. No octopus were removed from the reef but were present during the entire study, and two bat rays were observed near the reef, one before and one during the study.

Tagging.—Seventy adult abalone were purchased (\$38 each) from The Cultured Abalone, a commercial farm in Goleta, California. The average size of the abalone was 16.2 cm (max 17.9 cm, min 14.6 cm). These animals were used as broodstock on the farm and thought to be at least 10 years old. They were shipped in three batches to a holding facility in

San Pedro, California in moist foam and oxygen filled bags and held for up to thirteen days to tag, monitor, and reduce stress from transport. Upon arrival, the animals were measured, sexed, affixed with tags using Splash Zone marine epoxy or cyanoacrylate (Super Glue), and photographed. Of the 69 abalone tagged, 87% (60) were identified as female (Table 1). Since abalone are known for choosing crevices, ledges, and overhangs for their home scars multiple tags were used to make the identifiers visible from any angle. The tags identified which outplant batch the animal was from and had both a unique number identifier (Major Tag) and several auxiliary tags (Minor Tag). Each animal was given a "Major" tag with a number, a color coded zip tie, and up to four other "Minor" tags (Figure 1). The Major tags consisted of a 1.5 cm stainless steel disk with etched numbers; a 2.5 cm white plastic square with printed black numbers; or a 4 cm brass disk with printed black numbers. All of the abalone had a colored zip tie secured through the first or second respiratory pore. PIT (passive integrated transponder) tags were epoxied on the shells of 32 of the animals for the purposes locating the animals using a PIT tag reader. Minor tags consisted of one or more of the following: blue aluminum tree tags with etched numbers; colored plastic bottle caps; white plastic beads with black letters; red plastic key tags with white numbers; stainless steel washers; plastic chain links; and metallic painted plastic jewelry (shiny). No two animals had the same combination of tags. The white lettered beads were the only tag affixed with cyanoacrylate. Knowing that the abalone would be cryptic to the observing volunteer divers, the objects used for tagging were meant to help spot the animals and the combinations of tags helped to identify the animals in hard to see places.



FIGURE 1.—Example of multiple tagging methods for green abalone outplants illustrating "Major" and "Minor" tags. Recorded as Major tag: #33, Minor tags "E", yellow chain link, yellow zip tie, and PIT tag # (in the epoxy).

Following the tagging activity, the animals were placed in rectangular plastic milk crates and submerged in a recirculating seawater holding systems (18 °C) for up to 13 days. The top of the milk crate was covered with plastic mesh so the animals could not crawl out. There was one mortality while in the holding tanks presumably due to stress related to shipment.

Outplanting.—Sixty-nine green abalone were outplanted in three batches in May 2013, July 2013, and August 2013 (Table 1). The animals were monitored for survival for one year after the last outplanting (until August 2014). On the day of outplanting, the animals were checked for health and for any tag loss, the milk crates were put into large coolers with seawater from the holding tanks and transported to the outplant site by car and then by boat. They were in transport for approximately three hours. While on board the boat, fresh ocean water was exchanged with the water in the cooler by bucket. Divers descended to the reef with the milk crates. When on the bottom, the milk crates were turned on their side and four half-sized cinder blocks were zip- tied to each milk crate to weigh them down. The first and third outplant sites offered more ledges and overhangs while the second outplanting area was on the top of the reef just above the other two. All of the locations chosen to place the crates on were within 5 m of each other on the west end of the reef (Figure 2). In accordance with the outplanting permit, as many abalone as possible were recovered from the test site at the end of the study. All animals were measured at the beginning of the study and emergent animals were measured at the end of the study. Volunteers were asked to not share the outplanting location with anyone. Temperature loggers (Hobo) were deployed from 01 April 2013 to 25 March 2014.

Monitoring.—Monitoring began with the first outplanting in May 2013 and concluded one year after the last outplanting in August 2014, representing a 15-month study period. Rigorous monitoring was required to track the newly released animals as they were very mobile. In order to track this movement, the program utilized volunteers. In total, 28 volunteers were trained as abalone observers. Each dive was led by the Get Inspired project biologist and assisted by up to four other volunteer divers. During each dive, a diver was assigned a quadrant number within which to survey the reef for abalone. Every visible tag

Outplanted			
	Batch 1 5/26/13	Batch 2 7/22/13	Batch 3 8/11/13
Females	17	21	22
Males	2	1	6
Total	19	22	28
Survival 8/11/14			
Females	7	7	8
Males	1	0	5
Total	8	7	13

TABLE 1.—Proportion, by sex, of green abalone that were outplanted in three batches and their survival in Crystal Cove State Park, Orange County, California. Average size 16.2 cm.



FIGURE 2.—Map of the relative locations of the surviving abalone created 11 August 2014.

on the animal was recorded and the shells and tags were cleaned with a toothbrush to reduce encrusting organisms. By recording every observable tag, even if a "Major" tag could not be seen, the combinations of other visible tags usually lead to the positive identification of a specific animal. If an abalone could not be positively identified, it was not counted that day. Empty shells and shell fragments were also collected for positive identification.

Over the 15-month (60 week) study period, 64 monitoring dives (approximately 45 min each) were made totaling 260 dive hours. Dives were conducted after each outplanting every 48-hours for approximately two weeks to track the immediate movements of the animals. Monitoring tapered off from every 48-hours to every four days, then once per week, then once every 10 days by the end of the study period. Telescoping mirrors and flashlights were used to look under ledges and in deeper crevices for abalone. A map of the location of each abalone was created/updated after each monitoring dive. An animated map was created, at the end of the project, to illustrate relative movements of the animals throughout the study period. Survival was calculated by finding and counting the actual live animals that were positively identified at the end of the study period.

RESULTS

Site Survey.—The study site is composed of continuous rocky reef approximately 450 m² in size and surrounded by sand. The reef is composed of bedrock and roughly rectangular with dimensions approximately 18 m wide by 25 m long, with the highest point being approximately 2 m from the sand that surrounds it. Changes in contour are minimal

on the top of the reef with rugosity being less than 1 m. The south and north ends of the reef are composed of ledges, the west end gently slopes down toward the sand, the east end of the reef is a wall that drops 2.5 m vertically to the sand. The reef was at a relatively uniform depth of 8.4 m on the top of the reef and it slopes on each side to a maximum depth of 11.5 m to the sand on the east end. Due to sea urchin removal activities during a giant kelp restoration project conducted on the reef by the author 10 years earlier, sea urchin densities were low with lots of crevice and ledge space available.

Both site survey methods provided similar results with mature giant kelp (*Macrocystis pyrifera*) covering 10% of the reef providing a 30% canopy, reaching the surface over the reef. Approximately 15% of the reef was covered with pink crustose coralline algae, and articulated coralline algae covered 10% of the reef. Subtidal algae (*Cystoseira osmundacea*) covered 5% of the reef surface and other low lying red and brown alga covered 15% of the reef. The remaining 45% of the reef was occupied by sessile invertebrates including tunicates, bryozoans, worms (Serpulorbis sp.), gorgonians, anemones, and sponges. There were no wild abalone observed on this reef before outplanting. The average temperatures on the reef during outplating were as follows: May-18 °C, July-17.5 °C, August-15.8 °C

Tagging.—With continuous cleaning, the multiple tagging strategy worked well for the study period. Although the abalone routinely were wedged up and under rocks and ledges, the multi tag method allowed for identification of the animals from any angle. Only four of the major tags were lost due to poor epoxy application but the animals could still be identified by their minor tags. By the end of the project period, the brass tags (Major Tag) had tarnished making the numbers unreadable although we could still tell they were brass and coupled with the minor tags, each individual could still be identified. None of the zip ties or cyanoacrylate affixed tags were lost during the project period.

Monitoring and movements.—The milk crates allowed for the abalone to attach to something that could easily be moved, placed in a cooler, and transported to the study site with minimal stress to the animal. Upon release, most of the animals immediately moved out of the crates and even within the period of the dive (approximately 45 minutes) they moved up to 2 m away. All of the abalone left the milk crates within 48-hours of outplanting. Some made their immediate homes inside the cinderblocks that weighted down the milk crates so after the first outplanting batch we covered the cinderblocks so the abalone would be forced out onto the reef. All the abalone were released on the west end of the reef and subsequently 96% of the animals stayed on the west side of the reef within a 10 m radius of their release site, either under ledges or oriented at the sand reef interface during the project period. The farthest distance moved by an abalone was 44 m and the shortest distance moved was <1 m, both of which survived until the end of the project (Figure 2).

The PIT tag reader was only used once and was not effective at locating abalone during that one use. An animated map was created from each survey by compiling location information allowing us to see the relative movements of the animals over the course of the study. This animated map is available from the author.

Survival.—Mortality was closely associated with outplanting events with 61% of mortalities (17) occurring within the first 30 days of being outplanted and 9% (6) mortalities occurring in the first week of outplanting. Being out and on top of the reef (emergent) was not the key factor in mortality because several animals survived through the entire project while in conspicuous places on top of the reef. No direct predation was observed, although we did remove a giant sea star (*Pisaster giganteus*) from the shell of a live abalone. Thirteen mortalities were observed with crushed shells (Figure 3) and the meat gone, with the shell



FIGURE 3.-Example of crushed shells which resulted in 13 mortalities, predator unknown.

fragments found in the same location that the live animal had been previously observed. The shell crushing predator was never observed.

During this 15-month study period, 28 animals (40%) survived (Table 1). We searched adjacent reefs and boulders off the study site. Two abalone were found on a boulder 4 meters away from the outplant reef. They migrated there independently over a two month period. Another abalone ventured across 10 m of sand, across 20 m of reef, then across another 4 m of sand to another adjacent reef. There were 13 animals or 19% of the original 69 that were missing and not accounted for at the end of the project. Some of these animals presumably could have survived. Of the 13 missing animals, seven went missing within 30 days of outplanting and were never seen again. Three of those abalone were missing from the first week of outplanting.

After observing the habitat preferences of the first two batches of outplanted abalone, we chose the third outplanting site to match that of the first. It was 5 m away from the first on the edge of the west end of the reef with many overhangs and ledges. The last batch of abalone (28), outplanted in August, had 46% survival (Table 1). At the end of the 15-month period, eight abalone were retrieved in accordance with CDFW permits. The other 20 were not retrievable due to their positioning on the reef. The average growth of those eight surviving and retrieved abalone was 2.2 mm over the study period. Two of the 13 missing abalone were found dead two months after the end of the study period.

DISCUSSION

Based on findings from Tanaguchi et al. (2013), that green abalone expressed site infidelity when translocated; this survey site was specifically chosen because it was surrounded by sand. It was a disproven assumption that sand would act as a barrier and deter abalone movements. This finding presents a problem for future studies and may shed some light on previous studies where recapture rates were low. Green abalone will leave study sites even if it means crossing expanses of sand. It is possible more abalone emigrated from the survey site and these represent a proportion of the missing animals. Abalone movements and migrations are still poorly understood and continues to be a problem for abalone outplanting/reseeding efforts. Current telemetry will add new knowledge to this question.

Juvenile abalone of all species may move tens of meters, but this tendency decreases with age (Cox 1962, Tutschulte 1976). Adult abalone generally have very limited movements (Shepherd 1973, Tutschulte 1976). Abalone have been known to move considerable distances which has made previous restocking projects challenging and often ineffective (Shepherd 1986, Ault & DeMartini 1987, Tegner & Butler 1989). The majority of the abalone that survived until the end of this project appeared to move very little during the project period, though this also made them easier for divers to find repeatedly. After each survey, a map of the relative locations of the abalone was created. From this, we noted that 22 (79%) of the surviving abalone were within an 8 m radius of the release sights at the end of the project (Figure 2). Many did not appear to move at all from these scars during the entire study. This may be an advantage of using large adult green abalone. In a telemetry study, Coates et al. (2013) mentions a "flight" response when pink abalone were translocated, this was thought to occur within the first 20 days after moving the animals. The reported 61% of the abalone mortalities from this study, occurred in the first 30 days and may have been due to this "flight" response in the initial phase after outplanting.

The fact that the abalone used for this study were farm raised has not been shown to be a factor in their ability to hide (Tegner and Butler 1985, Schiel and Weldon 1987). It appears that abalone have home scars and possibly home ranges for localized movements (Ault & DeMartini 1987, Tutschulte and Connel 1988). Some of the abalone in this study found their home scars right away while others seemed to "roam" throughout the study period. The challenge is to determine how long it takes for introduced/outplanted large emergent adult abalone to get acclimated to their outplanted reef so they "settle" in fast and find a home scar. Ideally, it would be most advantageous to be able to place abalone directly onto their preferred home scar location in hopes that they would stay there when outplanted.

There were at least 13 known abalone mortalities which involved crushed shells and there were many more shell fragments found that could not be identified. Given that these abalone were large with a shell thickness of at least 3 mm, the list of possible predators was small. Very large bat rays and humans are capable of such crushing forces. Giant seabass are capable of both "sucking" them off the reef and inflicting the force necessary to crush the shells (L. Allen, California State University Northridge, personal communication). Often the crushed shell would be found with all the pieces in the same spot that the live abalone was seen just 48 hours before. In October 2013, suspecting poaching as the possible cause of the crushing mortalities, floating signs were posted around the reef warning humans that they were under surveillance and that they were violating the law by taking or killing the animals. It should be noted that within 30 days of the signs being put up, the crushing mortalities stopped. This could be coincidence. It should be noted, that in January 2014 a mortality event (sea star wasting disease), which affected the west coast of North America, resulted in a die-off of all sea star species observed on the reef (Hewson et al. 2014). Sea stars, therefore, were not a predator of concern during much of this study.

Difficulties involved in quantifying the results of outplanting and reseeding efforts make it difficult to make comparisons between studies (McCormick et al. 1994). A summary of abalone outplanting projects around the world, their duration, and percent survival was compiled by Chick et al. (2013). In comparison with those studies, this study has notable survival rates for the project duration (>1 year) and species outplanted, and also used the largest size abalone. Of the studies conducted with larger red and green abalone (40-100

mm) in southern California, survival rates were only as high as 2.8% and the researchers claimed they found no evidence of size differential in survival (Tegner and Butler 1985, Tegner and Butler 1989, Davis 1995). Although survival may be quantified using several different methods, it is important to note that the survival rates reported for this study are actual, not estimates. Each animal counted as a survivor was physically observed.

The frequency with which the animals in this study were surveyed was an advantage for monitoring their survival and it may have been the key to the high recapture rates. We were able to observe their movements regularly (at most every 10 days). With the success of tracking and survival of the animals in this study, it is evident that the strategy of using larger animals for restocking green abalone is worthy of further study. The survival rate for this project is notable and far exceeds survival rates in other studies with green abalone. The animals used in this study were estimated to be at least 10 years old (ranging in size from 14.6 cm to 17.9 cm) by the farmer from whom they were purchased. The costs associated with raising them to this size may be great but there have been decades of attempts to restock. One expensive project may be worth 30 or more failed larval or juvenile outplanting attempts. Perhaps, outplants could be clustered to create reproductive "colonies". The animals used in this study seem to be the largest used in a California abalone restocking/ outplanting study. We are currently spawning wild abalone to repeat this test in a future study in several different locations and may include animals 10 cm to 14 cm.

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