Endangered Species Act (ESA) Section 7(a)(2) Biological Opinion

Issuance of Scientific Research and Enhancement Permit 19571-2R to the NMFS Southwest Fisheries Science Center for Captive Research Activities on Black Abalone (*Haliotis cracherodii*), pursuant to Section 10(a)(1)(A) of the Endangered Species Act of 1973

NMFS Consultation Number: WCRO-2021-01839
ARN 151422WCR2021PR00022

Action Agency: Protected Resources Division, West Coast Region, NOAA National Marine Fisheries Service

Affected Species and NMFS' Determinations:

ESA-Listed Species	**Status**	**Is Action Likely to Adversely Affect Species?**	**Is Action Likely To Jeopardize the Species?**	**Is Action Likely to Adversely Affect Critical Habitat?**	**Is Action Likely To Destroy or Adversely Modify Critical Habitat?**
Black abalone (*Haliotis cracherodii*)	Endangered	Yes	No	No	NA
White abalone (*Haliotis sorenseni*)	Endangered	*No	NA	NA	NA

*Please refer to Section 2.12 for the analysis of species or critical habitat that are not likely to be adversely affected.

Consultation Conducted By: National Marine Fisheries Service, West Coast Region

Issued By: Chris E. Yates

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Date: *August 30, 2021*

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LIST OF ACRONYMS

BML	Bodega Marine Lab
CDFW	California Department of Fish and Wildlife
CICESE	Center for Scientific Research and Higher Education
COVID	Coronavirus disease
DQA	Data Quality Act
ELH	Egg Laying Hormone
ESA	Endangered Species Act
HAB	Harmful algal bloom
ITS	Incidental Take Statement
MARINe	Multi-Agency Rocky Intertidal Network
NMFS	National Marine Fisheries Service
NWFSC	Northwest Fisheries Science Center
OTC	Oxytetracycline
PIT	Passive integrated transponder
PRD	Protected Resources Division
SSC Pacific	Department of the Navy's SPAWAR Systems Center, Pacific
SWFSC	Southwest Fisheries Science Center
UC Davis	University of California, Davis
UCSB	University of California, Santa Barbara
UCSC	University of California, Santa Cruz
UV	Ultra-violet
UW	University of Washington
WCR	West Coast Region

1. INTRODUCTION

This Introduction section provides information relevant to the other sections of this document and is incorporated by reference into Sections 2 and 3 below.

1.1. Background

The National Marine Fisheries Service (NMFS) prepared the biological opinion (opinion) and incidental take statement (ITS) portions of this document in accordance with section 7(b) of the Endangered Species Act (ESA) of 1973 (16 USC 1531 et seq.), and implementing regulations at 50 CFR 402, as amended.

We completed pre-dissemination review of this document using standards for utility, integrity, and objectivity in compliance with applicable guidelines issued under the Data Quality Act (DQA) (section 515 of the Treasury and General Government Appropriations Act for Fiscal Year 2001, Public Law 106-554). The document will be available within two weeks at the NOAA Library Institutional Repository [https://repository.library.noaa.gov/welcome]. A complete record of this consultation is on file at the NMFS West Coast Region (WCR) Long Beach Office.

1.2. Consultation History

On December 16, 2020, the NMFS WCR Protected Resources Division (PRD) Permits Team received an application from the NMFS Southwest Fisheries Science Center (SWFSC) to renew their permit to "take"[1] endangered black abalone as part of ongoing captive holding and research activities at the SWFSC laboratory in La Jolla, California, and at captive facilities throughout California.

The Permits Team solicited public comments on the permit application from February 16 to March 18, 2021, via a notice published in the Federal Register (86 FR 9489; February 16, 2021). One public comment on the permit application was submitted. The commenter opposed the permit, stating that the permit has been in place for 50 years but has not made progress toward abalone recovery and indicating that specific groups have profited financially from the permit. The Permits Team notes that the permit has been in place for five years, not 50 years, and that issuing a permit does not provide government funding for the permitted work.

On July 29, 2021, the Permits Team developed the draft permit conditions and we initiated consultation. This consultation is on the proposal to issue Scientific Research and Enhancement Permit 19571-2R to the NMFS SWFSC, to authorize research and enhancement activities involving endangered black abalone. Issuance of the permit constitutes a Federal action that may affect marine species listed under the ESA.

This opinion analyzes the research and enhancement activities that may be authorized under Permit 19571-2R and evaluates their effects on ESA-listed resources, primarily endangered black abalone in captive facilities.

[1] Under the ESA, take means to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to do any of the preceding.

1.3. Proposed Federal Action

Under the ESA, "action" means all activities or programs of any kind authorized, funded, or carried out, in whole or in part, by Federal agencies (50 CFR 402.02).

The NMFS WCR PRD Permits Team proposes to issue Permit 19571-2R under the authority of Section 10(a)(1)(A) of the ESA to the NMFS SWFSC, to authorize research and enhancement activities for black abalone, as described in the permit application and summarized below. Permit 19571-2R would authorize researchers to continue most of the research and enhancement activities authorized under the current permit (Permit 19571). The proposed permit would authorize the research and enhancement activities for a period of five years.

The purpose of Permit 19571-2R is to support black abalone recovery efforts by developing successful spawning techniques, to reliably and consistently produce high quality juvenile black abalone for field restoration efforts and critical laboratory studies. The proposed permit activities focus on maintaining healthy black abalone and developing methods to condition, spawn, rear, and grow-out black abalone in captivity.

The final ESA Recovery Plan for Black Abalone (NMFS 2020) identifies captive propagation as an important tool to explore and develop in support of recovery efforts. The ability to produce black abalone in captivity would provide animals for critical laboratory research (e.g., early life history assessments, effects of oil/dispersants, methods to clean oiled abalone) without having to collect animals from the wild. It would also increase the feasibility of outplanting as an option to enhance wild populations, if needed for species recovery.

Research and enhancement activities under Permit 19571-2R would consist of four components:

(1) Maintain healthy captive populations of black abalone at approved facilities;
(2) Evaluate factors to improve reproductive conditioning of captive broodstock, including temperature, diet, photoperiod, seasonal tidal cycles, and reproductive hormones;
(3) Evaluate methods to induce spawning, including desiccation, thermal treatments, hydrogen peroxide, seasonal tidal cycles, and hormones; and
(4) Evaluate and optimize conditions for juvenile growth and survival, including temperature and diet.

Trained biologists will conduct the proposed research and enhancement activities. In the following sections, we describe each component in more detail and identify those aspects that are likely to affect ESA-listed resources.

We considered, under the ESA, whether or not the proposed action would cause any other activities that would have consequences[2] on listed species or their critical habitat, and we determined that it would not.

[2] A consequence is caused by the proposed action if it would not occur but for the proposed action and it is reasonably certain to occur.

1.3.1. Maintain Healthy Captive Populations

Black abalone are currently held at the SWFSC La Jolla Laboratory (n = 21), the California Department of Fish and Wildlife (CDFW) Shellfish Health Laboratory (n = 13), and the Monterey Bay Aquarium (n = 17). The captive animals include pre-listed abalone as well as individuals obtained after the ESA listing. Pre-listed abalone are those that were already in captivity at the time black abalone were listed as endangered under the ESA in 2009. Abalone obtained after the ESA listing include abalone that were confiscated in law enforcement cases as well as abalone that were collected as part of emergency response activities.

Permit 19571-2R would allow researchers to continue to maintain black abalone in captivity at approved facilities, as well as receive additional black abalone from the following sources:

- other facilities holding captive black abalone, including facilities in Mexico;
- law enforcement cases (most likely poaching) that involve confiscation of live black abalone;
- activities carried out, funded, or authorized by a Federal agency that involve removing black abalone from the wild (the effects of removal would be covered under a separate consultation for the Federal action); and
- emergency response activities that involve removing black abalone from the wild (e.g., rescues in response to spills, sedimentation events, vessel groundings).

The approved facilities include the SWFSC La Jolla Laboratory, the CDFW Shellfish Health Laboratory, and the Monterey Bay Aquarium. Additional facilities may be added to the list of approved facilities in the future.

At the approved facilities, abalone will be measured, weighed, tagged, and assessed for health and gonad condition. A genetic sample (epipodial clip, swab sample) will be collected using non-lethal methods. Abalone will be quarantined, tested for the pathogen that causes withering syndrome, and, if needed, treated with antibiotics according to established treatment protocols. Abalone will be assessed on a regular, minimal basis to limit handling. When appropriate, abalone may be integrated into broodstock conditioning and spawning experiments as described in Sections 1.3.2 and 1.3.3 below. In the event of mortalities, tissues will be preserved for analysis.

1.3.1.1. Transport and Holding

Black abalone (all life stages) may be transported to or between facilities by vehicle or by air, using established transport protocols to ensure appropriate conditions (e.g., temperature, oxygen levels). These transport protocols include placing the abalone in coolers with sufficient air and moisture and maintaining an appropriate temperature range. Transport times would be less than 24 hours and minimized as much as possible.

Researchers will maintain abalone under conditions that mimic natural conditions in the wild. Holding and husbandry will follow the general methods described in the White Abalone General Care Guide (Aquilino 2014). Captive facilities will use ambient filtered seawater and seawater delivery systems that maintain temperatures, supply oxygen, and remove waste products to keep

conditions optimal for abalone survival and growth. Some facilities may also treat the incoming seawater with ultra-violet (UV) light to remove pathogens. Researchers will daily monitor abalone health and holding conditions.

On a regular basis, researchers will remove animals from the substrate to measure their shell length, weight, and gonad condition. Generally, abalone will not be removed more than once per month, except during hormone experiments that may require more frequent handling (described below in Section 1.3.2 Broodstock conditioning studies and Section 1.3.3 Spawning induction studies). The standard method for removal is to slide a broad-faced plastic spatula or similar tool between the tank surface and the abalone foot. Alternate methods may be used for abalone that are difficult to remove (e.g., strongly hunkered down, attached to complex surfaces). These alternate methods include: exposure to 2-3% ethanol in seawater solution for 5-10 minutes; squirting of 25% ethanol in seawater along the shell base to encourage lifting of the shell such that a spatula can be inserted between the foot and tank surface; and exposure of abalone to a live seastar (*Pisaster* sp.) to encourage movement.

1.3.1.2. Tagging

Researchers will apply tags to the shell of each adult abalone for individual identification. Captive-bred juveniles may also be tagged once they reach an appropriate size. Two types of tags may be used: external visual tags and Passive Integrated Transponder (PIT) tags. Both types of tags will be attached to the shell with adhesive (e.g., Coraffix). External visual tags are typically small flat plastic tags with a unique color and number. These tags are small compared to the size of the shell and smaller than the size of organisms (e.g., barnacles, tubeworms) that typically grow on the shell of wild abalone. To attach, a small section of shell is cleaned and dried before applying the tag and glue. PIT tags are also glued to the shell, but typically to the underside of the shell just under where the mantle covers the shell surface. This placement facilitates nacre deposition over the PIT tag, to more permanently attach the tag to the shell. Individual abalone may be double tagged with both external visual tags and PIT tags, to account for tag loss or malfunction (e.g., of PIT tags).

1.3.1.3. Health monitoring, treatment, and analyses

Researchers will visually examine the captive animals to monitor their health. The three main health concerns for captive abalone are: (1) withering syndrome; (2) shell-boring organisms; and (3) parasitic sabellid polychaete worms.

Withering syndrome is a disease that causes the abalone's foot muscle to shrink and eventually results in death. All tanks holding black abalone will be tested on a regular basis for the presence of the pathogen that causes withering syndrome by collecting and analyzing fecal samples. Fecal samples may also be collected from individual abalone by inserting a flexible soft-tipped swab between the epipodium and mantle, along the gills, to evaluate whether individual abalone are infected with the pathogen. The swab sample will be transported to approved laboratories for analysis. If needed or recommended by the CDFW Shellfish Health Lab, abalone will be treated with oxytetracycline (OTC), an antibiotic capable of eliminating the pathogen from infected individuals. OTC treatment will follow the methods described in the "Abalone Disease and Parasite Management Protocol" (submitted as an attachment to the permit application). The

treatment involves immersing the abalone in an OTC bath solution. This treatment has been applied to captive abalone throughout California, including the black abalone currently held at the captive facilities, to successfully remove the pathogen with little negative effects on the abalone (Moore 2015).

Shell-boring organisms can infest and weaken the abalone's shell, leading to shell damage and potentially to death. Researchers will apply a wax treatment (Moore and Marshman 2015) to remove heavy infestations of shell-boring organisms. Wax treatment involves removing the abalone from the substrate, scrubbing the shell surface with a brush, and coating the shell surface with a wax mixture (beeswax and coconut oil), taking care not to cover the respiratory pores. Abalone are then returned to the holding tanks. The wax suffocates and kills the shell-boring organisms and eventually flakes off the shell on its own.

Parasitic sabellid polychaete worms can infest the growing edge of shells and cause shell deformity, slow growth, and brittleness. An eradication program has essentially removed the worms from farms and prevented new infestations. CDFW implements a sabellid-free certification program to monitor captive facilities and certify that animals have not been infested with sabellids. The SWFSC lab has already been certified as sabellid-free and undergoes regular inspections by CDFW. Inspections involve removing abalone from the holding tanks and visually inspecting individuals for the presence of sabellid worms. Depending on the number of animals at the facility, all or a subset of animals at each facility may be examined. Animals are out of the water for no more than 30 minutes. The shell waxing treatment may also be used to remove sabellid worms from the shell.

Permit 19571-2R will allow researchers to process, preserve, and analyze dead black abalone, as well as intentionally kill obviously sick/dying black abalone for necropsy. Obviously sick/dying individuals are those that show the following symptoms: reduction or cessation of feeding, extreme lethargy, withered and discolored foot muscle, and/or inability to adhere to the substrate. Abalone showing these symptoms will die soon. Pathologists recommend that obviously sick/dying animals be sacrificed and preserved before they die, so that the tissues remain intact for analysis to determine the cause of sickness/death. Once an abalone dies, the tissues deteriorate quickly and may no longer be useful for analysis.

Researchers will follow the procedures described in the White Abalone Moribund and Dead Animal Processing Guide (Moore 2014) to identify dead and obviously sick/dying abalone and sacrifice/preserve such individuals for analysis. Researchers may freeze whole animals, or dissect the relevant tissues (gut and foot muscle) and either freeze the tissues or fix them in formalin before placing in ethanol. Whole specimens, tissues, and parts will be analyzed at the SWFSC or at other approved facilities listed on the permit, including the University of Washington (UW), NMFS Northwest Fisheries Science Center (NWFSC), CDFW Shellfish Health Lab, CDFW Wildlife Forensic Lab, University of California Santa Cruz (UCSC) Shapiro Lab, and Center for Scientific Research and Higher Education (CICESE). Additional facilities may be added to the list of approved facilities.

1.3.1.4. Genetic sampling

Researchers will collect samples from juvenile and adult abalone for genetic analysis. Genetic analyses are needed to evaluate the genetic diversity and composition of the captive population and to inform genetic management of the captive population (e.g., plan crosses between individuals during spawning events to maximize diversity, track lineage in captive-bred stocks). Understanding the genetic makeup of the captive-bred population may provide a method to track the survival and reproduction of these individuals should they be released into the field (e.g., for field planting studies and population restoration efforts).

Researchers will obtain tissue samples from freshly dead individuals (see Section 1.3.1.3 Health monitoring, treatment, and analyses) or by taking epipodial clippings. Epipodial clipping is a well-established, non-lethal method to collect a tissue sample from abalone for genetic analysis (Hamm and Burton 2000). Researchers will use a pair of tweezers to grasp the end of one of the epipodial tentacles on the sides or posterior of the animal and cut the tentacle 1-2 millimeters from its base. Samples will be preserved (e.g., in 95-100% ethanol, RNAlater, or frozen) and sent to approved facilities, including the SWFSC, UW, NWFSC, CDFW, UCSC, and CICESE.

Researchers may also obtain genetic samples by swabbing the abalone shell or foot. Researchers will use the tip of a buccal swab to swab the surface of any exposed soft tissue or the shell of an individual abalone. Duplicate swabs will be collected for each abalone. Samples will be placed in vials filled with preservative solution (e.g., 70% or higher concentration of ethanol), if needed, and sent to approved facilities for analysis.

1.3.2. Broodstock Conditioning Studies

Researchers will evaluate the following factors to determine which support improved reproductive conditioning in adult black abalone: diet, holding temperature manipulation, photoperiod manipulation, artificial tidal cycles, and reproductive hormone injections. Researchers will evaluate broodstock condition by measuring the abalone's weight, size (shell length), and gonad index. These measurements will require removing the abalone from the tanks no more than once per month, except during hormone injection experiments which may require more regular handling (weekly for eight weeks). Researchers will limit the time out of the water, typically to less than 5 minutes.

Broodstock will primarily be fed brown kelps (*Macrocystis pyrifera* and *Egregia laevigata*). Researchers will supplement their diet with red algae (e.g., *Palmaria mollis*, *Gracilaria pacifica*) and green algae (e.g., *Ulva latica*) and also offer other native and non-native algal species encountered in the wild, to assess algal preferences and feed rates on different algal species. Broodstock will also be exposed to different photoperiod regimes and simulated tidal cycles to mimic natural conditions and seasonal cycles in the wild.

Short-term holding (1-2 months) at warmer temperatures (18-21˚C) appears to improve visual gonad indices, based on recent work on black abalone in Mexico (pers. comm with Fabiola Lafarga de la Cruz, CICESE, cited in the permit application). To evaluate this, researchers will conduct temperature studies by holding a subset of the broodstock at warmer temperatures (17, 19, and 21˚C) for a period of 1-3 months. All other broodstock will be maintained at 12-16˚C.

The SWFSC has been testing the use of reproductive associated hormones (e.g., GnRH) to improve gonad maturation and development in red abalone (*Haliotis rufescens*). Abalone are injected with hormones through the foot muscle. To evaluate the use of hormones on black abalone, researchers will conduct hormone studies by treating a subset of the black abalone broodstock with regular weekly hormone injections for eight weeks.

At the end of the three month period for temperature studies and the eight week period for hormone conditioning, researchers will assess broodstock condition by measuring weight, shell length, and gonad index. Researchers may also attempt to spawn the abalone, as visual gonad indices are not always correlated with spawning condition (see Section 1.3.3 Spawning induction studies). Researchers will coordinate temperature and hormone studies so that individual abalone are not subjected to more than two experimental trials per year.

1.3.3. Spawning Induction Studies

Researchers will use standard and modified methods to induce spawning in black abalone broodstock. Researchers will attempt to spawn broodstock a maximum of four times per year. Standard methods used to induce spawning in other abalone species include desiccation, thermal treatments, and hydrogen peroxide treatments. Researchers will also evaluate modified methods to induce spawning, including hormones and seasonal tidal cycles. During spawning experiments, abalone will be individually isolated in spawning buckets and monitored closely for signs of stress.

Standard spawning induction trials will generally follow the methods described in the White Abalone Spawning and Culturing Guide (Kawana and Aquilino 2020). These methods include placing male and female broodstock in separate containers, a desiccation period, and exposing them to increased temperatures and a solution of Tris-buffered seawater and hydrogen peroxide (6% H_2O_2). Once spawning occurs, or after about three hours of exposure to the Tris/H_2O_2 solution, the animals are removed from the solution and placed in filtered seawater. Any eggs and sperm released are collected and mixed to promote fertilization.

Researchers will evaluate whether spawning induction improves using extended desiccation periods and exposure to increased water temperatures ranging from 17-21˚C. Researchers will also evaluate the use of simulated tidal cycles and hormone treatments to induce spawning.

Seasonal tidal cycles may be important to induce spawning behavior, given that black abalone inhabit intertidal to shallow subtidal reefs. Researchers will vary the water level in black abalone holding tanks to simulate seasonal tidal cycles and variable periods of submersion similar to what black abalone would experience in their natural intertidal environment. During periods of greatest tidal range, the broodstock will be induced to spawn using the methods described in this section.

The SWFSC and CICESE (Lafarga lab) have successfully induced the release of eggs from black abalone using the Egg Laying Hormone (ELH) and APGW-amide. Under Permit 19571-2R, researchers will continue to evaluate ELH and APGW-amide, as well as other neuropeptide hormones used on abalone (e.g., whitnin, myomodulin, and FMRF-amide). Abalone will be

injected with hormones through the foot muscle prior to spawning attempts. Researchers will experiment with different dosage schedules. Past work involved an injection of APGW-amide followed by an injection of both APGW-amide and ELH about 24 hours prior to spawning attempts. Only broodstock with a gonad index of two or higher will be selected for hormone induction experiments. Individual abalone will not be subject to more than two experimental trials per year.

1.3.4. Juvenile Growth and Survival Studies

Permit 19571-2R will allow researchers to grow-out and maintain all captive-bred progeny produced during propagation activities at the approved facilities. Researchers will settle and rear captive-bred black abalone according to established protocols as described in the White Abalone Spawning and Culturing Guide (Kawana and Aquilino 2020). To determine survival rates through all stages of development, researchers will collect a sample at each stage to assess initial numbers (e.g., of released eggs, swimming trochophores, developing veligers) and enumerate settled veligers using a microscope. Researchers will also preserve a small number of each stage to document early life development.

Researchers will evaluate different temperatures and diets to optimize the growth of captive-bred juveniles. Juveniles will be exposed to different temperatures (17-21˚C) within the normal range for black abalone for 3-6 months. Diet studies will include different macroalgal species that juveniles would encounter in the wild, as well as artificial manufactured feed. Growth and survival of juveniles will be recorded on a regular (e.g., monthly) basis throughout the experiments.

1.3.5. Permit Conditions

Research permits lay out the conditions to be followed before, during, and after the research activities are conducted. These conditions are intended to: (a) manage the interaction between scientists and listed black abalone by requiring that research activities be coordinated between the permit holder and NMFS; (b) minimize effects on listed species; and (c) ensure that NMFS receives information about the effects the permitted activities have on the species concerned. NMFS will use annual reports to monitor the actual number of black abalone that are taken each year by scientific research and enhancement activities and will adjust permitted take levels if they are deemed to be excessive or if cumulative take levels rise to the point where they are detrimental to the listed species.

The proposed permit conditions refer to the following personnel under the permit: Permit holder, principal investigator, and co-investigator. “Permit holder” means the person, institution, or agency that is ultimately responsible for all activities of any individual who is operating under the authority of the permit. “Permit holder” refers to the permit holder or any employee, contractor, or agent of the permit holder. “Principal investigator” means the individual primarily responsible for the taking, importation, exportation, and any related activities conducted under the permit. “Co-investigator” means an individual who is qualified and authorized to conduct or directly supervise activities conducted under the permit without the on-site supervision of the Principal Investigator.

The proposed permit conditions include the following:

General Conditions

1. The Permit Holder must ensure that black abalone are taken only at the levels, by the means, in the areas, and for the purposes stated in the permit application, and according to the conditions in this permit.
2. The Permit Holder must not intentionally kill, or cause to be killed, any listed species unless the permit specifically allows intentional lethal take.
3. The Permit Holder must handle black abalone with care and provide adequate holding conditions for abalone health, including water temperatures within the range of black abalone, proper aeration and oxygen levels, and routine removal of waste products.
4. The Permit Holder must notify NMFS as soon as possible but no later than two days after any authorized level of take is exceeded or if such an event is likely. The Permit Holder must submit a written report, as described in Section B.5 of the permit, detailing why the authorized take level was exceeded or is likely to be exceeded.
5. The Permit Holder is responsible for all costs incurred by research and/or enhancement activities including determination of cause of death.
6. The person(s) actually doing the research must carry a copy of the permit while conducting the authorized activities.
7. The Permit Holder must allow any NMFS employee or representative to accompany personnel while they conduct the research activities.
8. The Permit Holder must allow any NMFS employee or representative to inspect any records or facilities related to the permit activities.
9. The Permit Holder may not transfer or assign this permit to any other person as defined in Section 3(12) of the ESA. This permit ceases to be in effect if transferred or assigned to any other person without NMFS' authorization.
10. NMFS may amend the provisions of the permit after giving the Permit Holder reasonable notice of the amendment.
11. The Permit Holder must obtain all other Federal, state, and local permits/authorizations needed for the research activities.
12. If the Permit Holder violates any permit condition, they will be subject to any and all penalties provided by the ESA. NMFS may revoke the permit if the authorized activities are not conducted in compliance with the permit and the requirements of the ESA or if NMFS determines that its ESA section 10(d) findings are no longer valid.

Duration of Permit

1. This permit expires on December 31, 2026. Researchers may conduct activities authorized by this permit through December 31, 2026. A renewal for this permit can be applied for through the NOAA Fisheries APPS website

(https://apps.nmfs.noaa.gov/index.cfm). A completed application must be submitted within six to nine months of the expiration date in order to be considered for the renewal without a break in coverage.

2. Unspecified mortality has been authorized under certain circumstances for the captive-origin progeny. These mortalities include death from natural causes, culling, and authorized research, which must be included in the annual report. In the event of an unusual mortality event in the captive facilities (mortality due to unique circumstances; e.g., disease outbreak, facility failure), research must be immediately suspended and all relevant protocols must be reviewed and, if necessary, revised to the satisfaction of NMFS. The Permit Holder must notify the NMFS contact listed on the first page of the permit within two days of the event. The Permit Holder must also submit a written incident report as described in Section B.5 of the permit. The Permit Holder, in consultation with NMFS, must re-evaluate the techniques that were used and those techniques must be revised accordingly to prevent further injury or death. NMFS may amend the permit in order to allow research activities to continue.
3. If authorized take or mortality is exceeded, the researchers must cease permitted activities and notify the NMFS contact listed on the first page of the permit as soon as possible, but no later than within two business days. The Permit Holder must also submit a written incident report as described in Section B.5 of the permit. NMFS may grant authorization to resume some or all permitted activities based on review of the incident report and in consideration of the Terms and Conditions of the permit.

Conditions Related to Captive Breeding and Research Activities

1. Researchers must comply with the following conditions related to the manner of taking and the methods of supervision, care, and transportation:
    a. Research activities are limited to those described in the application. Research topic areas include broodstock conditioning, spawning, larval rearing and settlement, juvenile survival and growth, early life stage development, captive holding, health and disease management, and genetics.
    b. Shell waxing may be conducted when necessary to prevent damage to the shell by boring organisms (e.g., when there is evidence of live *Polydora* covering the shell, or more than 50 percent of the surface of the shell shows evidence of boring organisms). Researchers should try to keep the animals moist and minimize the time they are kept out of water (the procedure should take less than 10 minutes).
    c. Broodstock abalone must be individually identifiable (e.g., by tagging).
    d. Prior to transferring black abalone to an approved facility for settlement and grow-out, educational purposes, or research, the responsible official of the facility must be designated as a co-investigator (CI) on the permit or possess a separate scientific research and/or enhancement permit.
    e. Prior to transfer to a new facility which has not previously held black abalone, husbandry and research protocols including disease screening and prevention of disease transmission at the facility must be submitted to NMFS for approval.

f. When transferring black abalone, the Permit Holder must follow best practices such as those described in the appendices to the White Abalone Recovery Plan (NMFS 2008).[3] The Permit Holder must handle the animals with care and provide a healthy environment, including appropriate temperature, oxygen, and water levels.

g. Public display of captively held animals is authorized provided that it is incidental to and does not interfere with attaining the survival or recovery objectives as described in the permit. Such incidental public display may only occur as part of an educational program. A portion of this program must describe the research and/or enhancement activities.

h. Researchers may euthanize obviously sick/dying animals to preserve them for necropsy to determine the cause of death. Obviously sick/dying individuals are those that show the following symptoms: reduction or cessation of feeding, extreme lethargy, withered and discolored foot muscle, and/or inability to adhere to the substrate. Abalone that fit this description are expected to die within days and may be preserved to determine the cause of death.

2. Researchers and approved facilities listed on the permit are authorized to transfer, receive, import, and export tissue samples, parts, whole live wild-origin animals or captive-origin progeny (e.g., embryos, larvae, juveniles, adults), including gametes, as well as dead black abalone for scientific research and enhancement activities. The ability to exchange live animals, dead specimens, and samples will facilitate collaboration among black abalone researchers in the U.S. and Mexico and enhance research in both areas. The Permit Holder must:

   a. Maintain a record of all live animals, dead specimens, parts, and tissue samples received from and transported to other facilities, including the purpose of the transfer, what was transferred (live animals, dead specimens, parts, tissue samples), origin (wild, captive, location), individual identifiers (e.g., tag numbers, cohort), transport methods, and final destination and disposition;

   b. Summarize these records in the annual report to NMFS; and

   c. Notify NMFS prior to importing/exporting live animals, dead specimens, parts, or tissue samples to/from approved co-investigators and approved facilities in Mexico.

3. Researchers and facilities listed on the permit may receive, maintain, and conduct permitted research and enhancement activities on wild-origin black abalone obtained through the following sources listed below. New individuals brought into the facilities must be quarantined, tested for the pathogen that causes withering syndrome, and, if needed, treated to remove the pathogen that causes withering syndrome.

   a. Captive black abalone held at other facilities, including facilities in Baja California, Mexico.

[3] See Appendix A: White abalone broodstock collection and holding protocol, *Pages A-1 to A-12 in*: NMFS. 2008. Final white abalone recovery plan (*Haliotis sorenseni*). Prepared by the White Abalone Recovery Team for NOAA NMFS Office of Protected Resources Division. 133 pp.

b. Law enforcement cases (e.g., poaching) that involve confiscation of live black abalone. In these cases, the black abalone would be under the custody of law enforcement and could be transferred to the captive facilities under the permit and placed on loan for research purposes. Permit 19571-2R would cover the receipt of and research and enhancement activities involving these black abalone once brought into captivity.

c. Activities carried out, funded, or authorized by a Federal agency that involve removing black abalone from the wild. Take associated with removing black abalone from the wild would be analyzed and covered by a consultation under Section 7 of the ESA for the action. Permit 19571-2R would cover the receipt of and research and enhancement activities involving these animals once brought into captivity.

d. Emergency response activities that involve removing black abalone from the wild (e.g., in response to a spill or landslide). Take associated with removing black abalone from the wild would be analyzed and covered under the appropriate ESA process for the action. Permit 19571-2R would cover the receipt of and research and enhancement activities involving these animals once brought into captivity.

4. Disposition: The Permit Holder is responsible for individuals resulting from captive breeding of ESA-listed species and all disposition alternatives are subject to the Terms and Conditions of the permit. For each year class, the Permit Holder must confer with NMFS on the proportion of individuals to be raised for each disposition option listed below. The following dispositions have been considered for the permit:

   a. Use in authorized research activities,

   b. Transfer to facilities for settlement and growout, educational purposes, and/or research activities, and

   c. Destroying.

5. Mortalities: Although unlikely, we consider the possibility that all of the wild-origin animals received and held in captivity under this permit could die in captivity, due to natural or unusual mortality events. See Section B.5 of the permit for further requirements.

<u>Number and Kind(s) of Protected Species, Location(s), and Manner of Taking</u>

1. The take table in the permit application outlines the number of black abalone that may be taken, and the locations, manner, and period in which they may be taken.

2. Researchers working under the permit may collect visual images (e.g., still photographs, motion pictures) as needed to document the permitted activities, provided the collection of such images does not result in takes of protected species.

3. The Permit Holder may use visual images collected under the permit in printed materials (including commercial or scientific publications) and presentations, provided the images are accompanied by a statement indicating that the activity was conducted pursuant to Permit No. 19571-2R. This statement must accompany the images in all subsequent uses or sales.

4. Upon written request from the Permit Holder, approval for photography, filming, or audio recording activities not essential to achieving the objectives of the permitted activities, including allowing personnel not essential to the research (e.g. a documentary film crew) to be present, may be granted by NMFS.
    a. Where such non-essential photography, filming, or recording activities are authorized, they must not influence the conduct of permitted activities or result in takes of protected species.
    b. Personnel authorized to accompany the Researchers during permitted activities for the purpose of non-essential photography, filming, or recording activities are not allowed to participate in the permitted activities.
    c. The Permit Holder and Researchers cannot require or accept compensation in return for allowing non-essential personnel to accompany Researchers to conduct non-essential photography, filming, or recording activities.
5. Biological Samples:
    a. The Permit Holder is responsible for all of the biological samples collected from listed species, including whole specimens, tissue samples, and shells. Such samples are subject to the Terms and Conditions of the permit.
    b. All biological samples collected from black abalone obtained under the permit shall be identified by a unique number and maintained according to accepted curatorial standards. After completion of initial research goals, any remaining samples or specimens shall be maintained by the Permit Holder or deposited into a bona fide scientific collection that meets the minimum standards of collection, curation, and data cataloging as established by the scientific community.
    c. The transfer of any biological samples from the Permit Holder to researchers other than those specifically identified in the application requires written approval from the NMFS. Any such transfer will be subject to such conditions as NMFS deems appropriate.
6. Commercial culture and sale of black abalone, including parts (e.g., shells), is forbidden.

Reports

1. The Permit Holder must submit annual, final, and incident reports, and papers or publications resulting from the research authorized herein to NMFS. Reports may be submitted:
    a. through the online system at https://apps.nmfs.noaa.gov, or
    b. by email attachment to the NMFS contact listed on the first page of the permit.
2. The Permit Holder must submit written incident reports related to serious injury and mortality events, or to exceeding authorized takes. The incident reports must be submitted to NMFS as soon as possible, but not more than two days from the occurrence of the incident. The incident report must include a complete description of the events and identification of steps that will be taken to reduce the potential for additional research-

related mortality or exceedance of authorized take.

3. The Permit Holder must submit an annual report to NMFS at the conclusion of each year for which the permit is valid. Annual reports are due by January 31st for the previous reporting year. Falsifying annual reports or permit records is a violation of the permit. Annual reports must describe the research and monitoring activities and include the following:

    a. Naturally-produced, wild-origin black abalone: the number of animals at each facility, the year obtained and their source (e.g., from enforcement cases, emergency response activities, or other facilities), and their health, survival, and spawning success;

    b. Captive-bred progeny: survival rates at each life stage and juvenile health and growth for each year class;

    c. A summary of biological samples, parts, and specimens collected and transferred among facilities, including those received from and transported to researchers in Mexico;

    d. A summary of mortalities, necropsy results, and determined causes of death;

    e. A description of research activities, their effects on the animals, and a brief summary of the research results; and

    f. A summary of progress toward developing a central repository for biological samples and toward developing a forum for sharing data and public outreach and education materials with the project partners.

4. The Permit Holder and researchers under the permit must develop a tracking system (e.g., database, spreadsheet) documenting the following information collected as part of the permit activities, to inform future analyses and implementation of the proposed captive activities. The Permit Holder must provide access to the tracking system to NMFS and the co-investigators and summarize the information in the annual reports.

    a. Tracking the source, survival, growth, and spawning success of wild-origin animals. The tracking system should include the following information: collection location and depth, collection date, collection purpose, holding facility, tag number, size, weight, gonad index, sex, genetic sample collected (epipodial clip or swab, sample number, date), health, growth, spawning success, fecundity, and crosses.

    b. Biological samples collected and analyzed. The tracking system should include the following information for each sample: collection date and location; name of collector; collection purpose; description of specimen (e.g., whole animal, epipodial sample, shell); life stage, origin (wild or captive), sex, size, weight, and tag number for the individual; and a summary of analysis results.

    c. Tracking captive breeding success of wild- and captive-origin broodstock. The tracking system should include the following information for each spawning event: date, facilities involved, number of broodstock involved, gonad index of broodstock, spawning success, gametes released, crosses produced, fertilization rate, larval survival, and juvenile survival.

    d. Tracking observations of disease and parasites and necropsy results. The tracking

system should include the following information for each facility: health monitoring results and necropsy results, including the following information for each specimen: date of death, origin (wild or captive), size, weight, age (if known), symptoms, description of specimen (preservation method, tissues), and cause of death.

5. The Permit Holder must submit a final report to NMFS within 90 days after expiration of the permit (March 31, 2027), or, if the research concludes prior to permit expiration, within 90 days of completion of the research.
6. Research results must be published or otherwise made available to the scientific community in a reasonable period of time, taking care to protect sensitive location data for abalone in the wild.

2. ENDANGERED SPECIES ACT: BIOLOGICAL OPINION AND INCIDENTAL TAKE STATEMENT

The ESA establishes a national program for conserving threatened and endangered species of fish, wildlife, plants, and the habitat upon which they depend. As required by section 7(a)(2) of the ESA, each Federal agency must ensure that its actions are not likely to jeopardize the continued existence of endangered or threatened species, or adversely modify or destroy their designated critical habitat. Per the requirements of the ESA, Federal action agencies consult with NMFS and section 7(b)(3) requires that, at the conclusion of consultation, NMFS provide an opinion stating how the agency's actions would affect listed species and their critical habitats. If incidental take is reasonably certain to occur, section 7(b)(4) requires NMFS to provide an ITS that specifies the impact of any incidental taking and includes reasonable and prudent measures (RPMs) and terms and conditions to minimize such impacts.

The proposed action is likely to adversely affect endangered black abalone. We analyze these effects on black abalone in this opinion. Although critical habitat has been designated for black abalone, the proposed action and its effects will not occur in or affect designated critical habitat.

Although the proposed research and enhancement activities will occur at facilities that also hold endangered white abalone (*Haliotis sorenseni*), the proposed action is not likely to adversely affect white abalone. We summarize our analysis regarding effects of the proposed action on white abalone in Section 2.12 ("Not Likely to Adversely Affect" Determinations).

2.1. Analytical Approach

This biological opinion includes a jeopardy analysis. The jeopardy analysis relies upon the regulatory definition of "jeopardize the continued existence of" a listed species, which is "to engage in an action that reasonably would be expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of that species" (50 CFR 402.02). Therefore, the jeopardy analysis considers both survival and recovery of the species.

The 2019 regulations define effects of the action using the term "consequences" (50 CFR 402.02). A consequence is caused by the proposed action if it would not occur but for the proposed action and it is reasonably certain to occur. As explained in the preamble to the regulations (84 FR 44977), this definition does not change the scope of our analysis and in this opinion we use the terms "effects" and "consequences" interchangeably.

We use the following approach to determine whether a proposed action is likely to jeopardize listed species:

- Evaluate the rangewide status of the species expected to be adversely affected by the proposed action.
- Evaluate the environmental baseline of the species.
- Evaluate the effects of the proposed action on species and their habitat using an exposure-response approach.
- Evaluate cumulative effects.
- In the integration and synthesis, add the effects of the proposed action and cumulative effects to the environmental baseline, and, in light of the status of the species, analyze whether the proposed action is likely to directly or indirectly reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of that species.
- If necessary, suggest a reasonable and prudent alternative to the proposed action.

2.2. Rangewide Status of the Species

This opinion examines the status of each species that would be adversely affected by the proposed action. The status is determined by the level of extinction risk that the listed species faces, based on parameters considered in documents such as recovery plans, status reviews, and listing decisions. This informs the description of the species' likelihood of both survival and recovery. The species status section also helps to inform the description of the species' "reproduction, numbers, or distribution" as described in 50 CFR 402.02.

Two factors affecting the rangewide status of black abalone are climate change and ocean acidification. Climate change effects may increase susceptibility to disease, reduce kelp growth, and alter the distribution of rocky intertidal habitat along the coast. Ocean acidification can affect reproduction, development, growth, and survival of black abalone, as well as the growth of important algal species. In the sections below, we discuss the potential effects of climate change and ocean acidification in more detail.

2.2.1. Rangewide Status of Black Abalone

Black abalone are marine snails with one shell, typically 5 to 9 open respiratory pores, an anterior head, and a large muscular foot used for movement as well as to clamp down onto hard substrates to avoid being dislodged by wave action (Cox 1960). Black abalone occupy rocky habitats from the upper intertidal to 6 meters depth. Historically, black abalone occurred from Crescent City (Del Norte County, California) to southern Baja California (Geiger 2004), but the current range is from Point Arena, California, to Bahía Tortugas, Mexico (74 FR 1937, 14

January 2009). Black abalone are most commonly observed in the middle and lower intertidal, in habitats with complex surfaces and deep crevices that provide shelter for juvenile recruitment and adult survival (Leighton 1959, 2005; Cox 1960; Leighton and Boolotian 1963; Douros 1985, 1987; Miller and Lawrenz-Miller 1993; VanBlaricom et al. 1993; Haaker et al. 1995). They are able to withstand extreme variations in temperature, salinity, moisture, and wave action, and are usually strongly aggregated, with some individuals stacking two or three on top of each other (Cox 1960; Leighton 2005).

Abalone are broadcast spawners; individuals release their gametes into the water column and rely on external fertilization. Thus, abalone must be in close enough proximity to one another to reproduce successfully. Abalone also have a short planktonic larval stage (about 3-10 days) before settlement and metamorphosis (McShane 1992). Larval black abalone are believed to settle on rocky substrate with crustose coralline algae, which serves as a food source for post-metamorphic juveniles, along with microbial and diatom films (Leighton 1959; Leighton and Boolotian 1963; Bergen 1971). Reproductive maturity is reached at a size of about 50 mm shell length in females and about 40 mm in males (Leighton 1959; Ault 1985). Spawning has not been observed in the wild, but likely occurs from spring to early autumn (Leighton 1959, 2005; Leighton and Boolotian 1963; Webber and Giese 1969) and may extend into winter (VanBlaricom et al. 2009).

2.2.1.1. Population Trends

Based on fisheries and long-term monitoring data since the 1970s, black abalone are believed to be naturally rare at the northern (north of San Francisco) (Morris et al. 1980) and southern (south of Punta Eugenia; P. Raimondi, pers. comm., cited in VanBlaricom et al. 2009) extremes of the species' range. Areas of highest abundance occurred south of Monterey, particularly at the Channel Islands off southern California (Cox 1960; Karpov et al. 2000). Rogers-Bennett et al. (2002) estimated a baseline abundance of 3.54 million black abalone in California, based on landings data from the peak of the commercial and recreational fisheries (1972-1981). This estimate provides a historical perspective on patterns in abundance and a baseline against which to compare modern day trends. We note, however, that black abalone abundances in the 1970s to early 1980s had reached extraordinarily high levels, particularly at the Channel Islands, possibly in response to the elimination of subsistence harvests by indigenous peoples and large reductions in sea otter populations. Thus, our understanding of black abalone abundance and distribution for this time period may not accurately represent conditions prior to commercial and recreational harvest of black abalone in California.

Beginning in the mid-1980s through the 1990s, black abalone populations declined dramatically due to the spread of withering syndrome (Tissot 1995), a disease caused by a pathogen that affects the animal's digestion and causes starvation leading to foot muscle atrophy, lethargy, and death (Friedman and Finley 2003; Braid et al. 2005). Withering syndrome results in rapid (within a few weeks) and massive (reductions of over 80%) mortalities in affected populations (Neuman et al. 2010). Overall, populations throughout southern California and as far north as Cayucos declined in abundance by more than 80%; populations south of Point Conception declined by more than 90% (Neuman et al. 2010). Commercial and recreational harvest of black abalone contributed to some degree, but the primary cause of these declines was withering syndrome.

The disease has also affected populations in Baja California, but little is known about the species' status in Mexico.

Populations north of Cayucos have not yet exhibited signs of the disease, but all are likely infected by the pathogen. Abalone may be exposed to and infected by the pathogen without showing symptoms; however, once symptoms develop, the animals rapidly succumb to death (Friedman et al. 1997a, 2000, 2002). The pathogen has been detected in all coastal marine waters off southern California to Sonoma County and at Southeast Farallon Island (Moore et al. 2002; Friedman and Finley 2003) (pers. comm. with Jim Moore, CDFW, 20 November 2015; pers. comm. with Jim Moore, CDFW, cited in VanBlaricom et al. 2009).

Most black abalone populations affected by withering syndrome remain at low densities, below the estimated levels needed to support successful reproduction and recruitment (e.g., 0.34 abalone per m^2) (Neuman et al. 2010). Populations not yet affected by the disease (north of Cayucos) have densities greater than this threshold value (range: 1.1 to 10.5 abalone per m^2), whereas populations affected by the disease (south of Cayucos) have densities below this threshold value (range: 0 to 0.5 abalone per m^2) (Neuman et al. 2010).

Despite these low densities, researchers have observed evidence of recent recruitment and increases in abundance at several locations throughout southern California, including the Palos Verdes Peninsula, Laguna Beach, Santa Cruz Island, San Miguel Island, and San Nicolas Island (Richards and Whitaker 2012; Eckdahl 2015; unpublished data by Glenn VanBlaricom, U.S. Geological Survey, 22 June 2015). These observations for black abalone, and similar observations for other abalone species in California, indicate that we need to consider additional factors when assessing population viability. Recent studies also indicate the potential for disease resistance in wild black abalone populations. A bacteriophage has been discovered that infects the pathogen, reduces its lethal effects, and improves the survival of infected abalone (Friedman and Crosson 2012; Friedman et al. 2014; Crosson et al. 2014). Genetic-based disease resistance may also exist and is the subject of ongoing studies at the UW (VanBlaricom et al. 2009).

2.2.1.2. Additional Range-wide Threats

The relative effect of illegal harvest on black abalone status and recovery is poorly understood and requires further evaluation. In 2020, CDFW and researchers throughout the California coast noted an increase in the number of people visiting the rocky intertidal and harvesting or temporarily removing invertebrates (CDFW 2020) (John Ugoretz unpublished observations, Multi-Agency Rocky Intertidal Network (MARINe) meeting, 3 October 2020). This increase in human use activities poses a direct threat to black abalone populations because of the potential increase in illegal harvest as well as trampling of intertidal habitats. The increased harvest activities may also indirectly affect black abalone by altering the invertebrate community.

Elevated water temperatures resulting from local discharges, warm water events, and climate change could exacerbate disease effects on black abalone. Disease transmission and manifestation is intensified when local sea surface temperatures increase by as little as 2.5 ºC above ambient levels and remain elevated over a prolonged period of time (i.e., a few months or more) (Friedman et al. 1997b; Raimondi et al. 2002; Harley and Rogers-Bennett 2004; Vilchis et al. 2005). The disease appears to progress northward along the coast with increasing coastal

warming and El Niño events (Tissot 1995; Altstatt et al. 1996; Raimondi et al. 2002), and poses a continued threat to the remaining healthy populations. In 2015-2016, researchers observed increased numbers of diseased individuals at long-term monitoring sites, likely due to warmer water conditions (pers. comm. with Karah Ammann, UCSC, on 8 March 2016). It is not yet known how elevated water temperatures may affect the bacteriophage and genetic resistance.

Climate change and ocean acidification may also have range-wide effects on black abalone. In addition to increasing susceptibility to disease, warming ocean temperatures could reduce the growth of macroalgae (an important food source for black abalone) and shift the distribution of black abalone if temperatures in the southern part of the range increase above the optimal range. Sea level rise could alter the distribution and availability of rocky intertidal habitat. Black abalone may be able to adapt to changes in their habitat conditions, depending on the time frame over which these changes occur, but some populations and habitats may be lost. Increases in the frequency, severity, and extent of wildfires as a consequence of climate change also affect black abalone and their habitat. For example, severe wildfires burned along the central California coast in August 2020, followed by an atmospheric river rain event in January 2021, resulting in massive debris flows that buried large expanses of rocky intertidal habitat and black abalone populations. Researchers are evaluating the effects of this debris flow event on black abalone at the population and species level, as well monitoring how the affected populations recover.

Ocean acidification could hinder normal growth, development, and survival of black abalone by altering pH levels, carbonate availability, and the growth of crustose coralline algae (an important component of juvenile settlement habitat) (Crim et al. 2011). Studies on other abalone species indicate varying effects depending on the species, life stage, the degree to which pH levels decrease, and the presence of other stressors. Potential effects of ocean acidification on black abalone include reduced reproduction, abundance, and recruitment. Studies specific to black abalone are needed to evaluate the potential effects on different life stages and under multiple stressors.

2.2.1.3. Overall Status and Recovery Needs

Black abalone face high risk in each of four demographic risk criteria: abundance, growth rate and productivity, spatial structure and connectivity, and diversity (VanBlaricom et al. 2009). Although we know withering syndrome has affected populations in Baja California, little information exists regarding the species' status in that portion of the range. Long-term monitoring data in California indicates that populations affected by the disease remain at low abundance and density. The declines in abundance have potentially resulted in a loss of genetic diversity, though this needs to be evaluated. Although some sites in southern California have shown evidence of recruitment, natural recovery of severely-reduced populations will likely be a slow process. Illegal harvest is a concern, particularly in areas with relatively easy public access. Withering syndrome and other diseases continue to pose a threat to the remaining healthy populations (Raimondi et al. 2002; NMFS 2020). Elevated water temperatures and ocean acidification are range-wide threats that have the potential to exacerbate disease effects, reduce habitat quality and availability, and reduce the survival, growth, and development of black abalone. In addition, emergency events such as oil spills, landslides, and debris flows can affect large stretches of coast and result in the loss of populations.

Recovering the species will involve protecting the remaining healthy populations and increasing the abundance and density of populations that have declined, while also finding practical ways to address ongoing and emerging threats. Continued long-term monitoring will be critical to track and evaluate the species' status and the progression of withering syndrome along the coast. We must also raise the public's awareness, improve enforcement of protections, and evaluate the feasibility and effectiveness of recovery tools, such as habitat restoration, local aggregation, translocation, and captive propagation and outplanting. The research and enhancement studies in this proposed permit would directly address the evaluation of captive propagation as a recovery tool, as well as inform efforts to protect and restore populations and address threats.

2.3. Action Area

"Action area" means all areas to be affected directly or indirectly by the Federal action and not merely the immediate area involved in the action (50 CFR 402.02).

The action area for this consultation consists of land-based facilities throughout the U.S. West Coast where captive black abalone will be maintained, propagated, and subjected to research and enhancement activities, as well as laboratories where specimens or samples will be analyzed. These facilities and laboratories within the action area are connected through their transit routes. The proposed permit includes the following approved facilities for holding live black abalone: the SWFSC La Jolla Lab, the CDFW Shellfish Health Lab, and the Monterey Bay Aquarium. The proposed permit also includes the following approved facilities for receiving and analyzing specimens, tissues, and parts: the SWFSC La Jolla Lab, UW, NWFSC, CDFW Shellfish Health Lab, CDFW Wildlife Forensic Lab, UCSC Shapiro Lab, and CICESE. Additional facilities may be added to the list of approved facilities upon request by the Permit Holder, if the facilities meet the permit conditions.

2.4. Environmental Baseline

The "environmental baseline" refers to the condition of the listed species in the action area, without the consequences to the listed species caused by the proposed action. The environmental baseline includes the past and present impacts of all Federal, State, or private actions and other human activities in the action area, the anticipated impacts of all proposed Federal projects in the action area that have already undergone formal or early section 7 consultations, and the impact of State or private actions which are contemporaneous with the consultation in process. The consequences to listed species from ongoing agency activities or existing agency facilities that are not within the agency's discretion to modify are part of the environmental baseline (50 CFR 402.02).

In this environmental baseline, we focus on the effects of past research and enhancement activities on captive black abalone within the action area. These effects include past captive spawning, early development, and research studies, as well as past disease studies and analyses.

2.4.1. Captive Spawning, Early Development, and Research Studies

Previous attempts to spawn black abalone in captivity have largely been unsuccessful. To date, we know of only one documented successful spawning of black abalone in captivity, for early

life development and thermal studies conducted by TERA Corporation in the 1970s (Pacific Gas & Electric Company 1982). Researchers were able to induce spawning in black abalone using several hours of desiccation followed by exposure to UV-irradiated seawater at about 18°C. The studies found that early larval development was normal at temperatures between 10 to 22°C.

The 21 black abalone currently held at the SWFSC La Jolla Lab include 16 abalone that were originally collected in 2006-2008 by researchers at the University of California, Santa Barbara (UCSB) (prior to the species' listing under the ESA in 2009), as well as 5 abalone that were rescued in response to a landslide in 2017. The abalone collected by UCSB researchers were originally held at UCSB. In 2013, 12 of these black abalone were transferred from UCSB to the Department of the Navy's SPAWAR Systems Center, Pacific (SSC Pacific), Environmental Sciences Division in San Diego. In August 2013, NMFS issued Scientific Research Permit 17405 to Dr. David Lapota at the SSC Pacific to develop captive breeding methods for black abalone using these animals. Due to unforeseen circumstances, the Navy could not continue the abalone research program at the SSC Pacific and transferred the black abalone to the SWFSC La Jolla Lab in December 2014. Of the original 12 black abalone transferred from UCSB, two died while at the SSC Pacific facility, due to disease (Lapota 2015). Two more died about two months after transfer to the SWFSC La Jolla Lab, most likely due to health issues (pers. comm. with Paula Sylvia, SWFSC, on 4 April 2015). In December 2016, the remaining black abalone at UCSB (n = 8) were transferred from UCSB to the SWFSC La Jolla Lab.

In November 2017, nine black abalone were transferred to the SWFSC La Jolla Lab from the Mud Creek field site. These black abalone were collected as part of rescue efforts in response to the Mud Creek landslide in 2017. The nine black abalone were categorized as severely injured and not likely to survive if relocated to another field site. Instead, they were transferred to the SWFSC La Jolla Lab for rehabilitation and to serve as broodstock for captive spawning studies. Three of these black abalone died in 2017 shortly after arrival at the SWFSC La Jolla Lab, likely due to injuries sustained during collection. An additional black abalone died in 2020 due to an unprecedented red tide event that resulted in poor water quality at the SWFSC La Jolla Lab (SWFSC 2021).

Under Permit 19571, SWFSC researchers conducted six spawning attempts between January 2017 and December 2019 (SWFSC 2021). Researchers successfully induced spawning in ripe female black abalone using a combination of extended desiccation periods and hormone injections (synthetic peptides APGW-amide and abalone ELH). Researchers were able to reliably induce ripe females to release eggs in two spawning attempts; however, the number of eggs released was much lower than expected. Researchers also evaluated methods to improve reproductive conditioning. Food preference studies indicate black abalone prefer brown algae (*Macrocystis pyrifera* and *Egregia laevigata*). Seasonal temperature variations (11-16˚C) and photoperiod manipulations did not produce a measurable improvement in visual gonad index scores. Individual abalone that had higher scores maintained high scores and vice versa.

Black abalone are also held in captivity at the CDFW Shellfish Health Lab and the Monterey Bay Aquarium. The CDFW Shellfish Health Lab currently holds 13 black abalone. These include nine that were originally collected in the late 1980s from sites that include Año Nuevo Island, Carmel area, Santa Rosa Island, and Vandenberg Air Force Base. In July 2016, an

additional six black abalone (originally collected from Carmel) were transferred from Dr. Carolyn Friedman's lab at the University of Washington. In 2020, two of the black abalone at the CDFW Shellfish Health Lab died. At this time, researchers at the CDFW Shellfish Health Lab do not plan to use the abalone in any active research projects, other than regular health assessments and genetic sampling.

The Monterey Bay Aquarium currently holds 17 black abalone obtained from CDFW enforcement confiscations. The black abalone are held in two separate exhibits at the Monterey Bay Aquarium. At this time, the black abalone are not used in any active research projects. The Monterey Bay Aquarium staff conduct regular health assessments.

2.4.2. Disease Studies and Analyses

Withering syndrome is an ongoing threat to black abalone populations in the wild as well as in captivity. As described in section 2.2.1 (Rangewide Status of Black Abalone), recent work has led to the discovery of a bacteriophage that reduces the pathogenicity of the WS pathogen and improves the survival of infected abalone when exposed to elevated water temperatures (Friedman and Crosson 2012; Friedman et al. 2014; Crosson et al. 2014). Studies are ongoing to evaluate the distribution of the bacteriophage along the coast and its role in reducing the threat of withering syndrome to black abalone (pers. comm. with Carolyn Friedman, UW, 13-15 July 2015). To minimize the risk of an outbreak among captive populations, infected animals are held at cool water temperatures and/or treated with OTC and held in UV-treated water. Researchers also regularly monitor the health of the captive black abalone populations and maintain appropriate water temperatures to limit disease transmission and disease-induced mortality (Ben-Horin et al. 2013).

Other abalone diseases (e.g., Herpes virus, Vibrio) have emerged over the past several decades and pose a potential risk to black abalone. To date, no outbreaks have been observed in wild or captive black abalone populations in California, but great care is needed to closely monitor and manage potential pathways through which pathogens and invasive species can be introduced (e.g., import, transfer for aquaculture, research, food and hobby markets).

2.5. Effects of the Action

Under the ESA, "effects of the action" are all consequences to listed species that are caused by the proposed action, including the consequences of other activities that are caused by the proposed action. A consequence is caused by the proposed action if it would not occur but for the proposed action and it is reasonably certain to occur. Effects of the action may occur later in time and may include consequences occurring outside the immediate area involved in the action (see 50 CFR 402.17). In our analysis, which describes the effects of the proposed action, we considered 50 CFR 402.17(a) and (b).

We use the "exposure-response-risk" approach to analyze the effects of the proposed action on black abalone. First, we evaluate the exposure of individual black abalone to the effects of the action. Next, we evaluate how individual black abalone are likely to respond to those effects. We then evaluate how those responses are expected to reduce an individual's fitness (i.e., growth, survival, annual reproductive success, and lifetime reproductive success). Finally, we evaluate

the risk to black abalone at the individual, population, and species level, to determine whether the proposed action could appreciably reduce the species' likelihood of survival and recovery in the wild.

In our analysis of effects, we consider the proposed permit conditions described under Section 1.3.5 (Permit Conditions) and their effectiveness at reducing adverse effects on black abalone. We expect the Permit Holder to comply with the proposed permit conditions, because the Permit Holder complied with all of the permit conditions under the current permit (19571).

2.5.1. Effects of the Action on Black Abalone

Permit 19571-2R would authorize research and enhancement activities that involve direct take of naturally produced (wild-origin) and captive-bred black abalone. Activities involve measuring, swabbing, tagging, and handling individual black abalone, as well as the collection of epipodial clips, samples of early life stages, and dead and obviously sick/dying individuals. Table 1 summarizes the annual take of black abalone that would be allowed under Permit 19571-2R.

Table 1. Proposed annual take of black abalone under Permit 19571-2R.

Life Stage	Origin	Research Component	Take Activity	Number of Individuals	Estimated Mortality
Adult	Wild	Maintain existing captive black abalone; broodstock conditioning and spawning induction studies	Captive, maintain, monitor, breed, lab experiments; Tagging; Tissue Sample; Mortality; Transport	51[a]	51[d]
Adult, juvenile	Wild	Receive additional black abalone from various sources; maintain in captivity; broodstock conditioning and spawning induction studies	Captive, maintain, Monitor, breed, lab experiments; Tagging; Tissue Sample; Mortality; Transport	200[b]	200[d]
Egg, Larval, Juvenile	Captive	Maintain captive-bred black abalone; document early life survival and development; juvenile growth and survival studies	Captive, maintain; monitor; lab experiments; Tagging; Tissue Sample; Mortality; Transport	Unlimited[c]	Unlimited[d]

[a] Currently, a total of 51 black abalone are held at the SWFSC La Jolla Lab (n=21), the CDFW Shellfish Health Lab (n=13), and the Monterey Bay Aquarium (n=17).

[b] Permit 19571-2R would authorize researchers and approved facilities to maintain and conduct permitted activities on an additional 200 black abalone received from law enforcement cases, Federal actions, and emergency response activities.

[c] Permit 19571-2R would authorize researchers and approved facilities to maintain and conduct permitted activities on all captive-bred black abalone produced under this permit. This can include millions of eggs and larvae and thousands of juveniles per year.

[d] Mortalities: Although unlikely, we consider the possibility that all of the black abalone held in captivity under this permit could die in captivity, due to natural mortality or unusual mortality events.

The SWFSC La Jolla Lab, CDFW Shellfish Health Lab, and Monterey Bay Aquarium currently hold a combined total of 51 wild-origin black abalone. Permit 19571-2R would allow the approved facilities to receive an additional 200 wild-origin black abalone that become available

through law enforcement cases, emergency response activities, and Federal agency actions that involve live black abalone. Permit 19571-2R would allow the approved facilities to receive, maintain, and use these animals in captive research and enhancement activities.

Permit 19571-2R would also allow the approved facilities to retain all captive-bred black abalone produced under this permit. The main goal of this research is to develop reliable methods to captively propagate black abalone, to produce healthy animals for future research and field planting efforts to support black abalone recovery. Therefore, Permit 19571-2R would not restrict the number of captive-bred black abalone that can be produced. Permit 19571-2R would allow for unlimited take and mortality of captive-bred black abalone of all life stages. Each year, researchers could produce and handle millions of eggs and larvae and thousands of juveniles.

2.5.1.1. Holding and Grow-out

Permit 19571-2R would allow researchers at approved facilities to hold and grow-out both wild-origin and captive-bred black abalone of all life stages. Animals will be held in land-based facilities and handled on a regular basis to measure, weigh, and assess their health. Researchers will optimize holding conditions and implement best practices for general health and husbandry, such as maintaining water quality, water temperature, regular feeding, and regular tank cleaning. As part of the proposed permit activities, researchers will evaluate optimal conditions for black abalone survival and growth at each life stage.

We expect removal and handling to cause minor, temporary stress to individual black abalone. Removal from the substrate also has the potential to cause minor injuries (e.g., cuts to the foot muscle). Researchers will implement measures to minimize stress and injury. First, removal and handling will be conducted no more than once per month. Second, researchers will minimize injury by carefully removing abalone from the holding tanks by hand or by using a plastic spatula and/or abalone iron. When needed, researchers will use ethanol to sedate the abalone prior to removal. Ethanol has been used successfully to sedate small juveniles with minimal harm to the individuals (pers. comm. with Kristin Aquilino, University of California, Davis (UC Davis) Bodega Marine Lab (BML), on 1 April 2016). Sedation minimizes the potential for injury to the foot muscle or for cracking the shells when removing small animals. When measuring, weighing, and assessing health and gonad development, researchers will limit the time the animals are out of the water to a few minutes (less than five minutes) and minimize contact with soft tissues as much as possible.

We expect abalone to experience natural mortality across all life stages. For early life stages (embryo through newly-settled juveniles), natural mortality can be as high as 100 percent (unpublished data by Kristin Aquilino, BML, on 20 January 2016). After about one-year of age, expected natural mortality rates drop to about 5 to 10 percent or less, based on data from the white abalone captive breeding program (NMFS 2011). A catastrophic event, such as a system failure or disease outbreak, could kill all of the captive abalone; however, we believe the likelihood of a catastrophic event is low. Researchers will implement best practices for holding, husbandry, and disease and health management, including disease monitoring and treatment and daily monitoring of seawater flow and temperatures. To document early life survival and development (embryo through newly-settled juveniles), researchers will collect and preserve a small sample of individuals. The number collected would be a very small proportion of the

numbers we expect to be produced at each life stage (millions of eggs and larvae; thousands of juveniles).

2.5.1.2. Transport

Permit 19571-2R would allow researchers to transport abalone between facilities by vehicle or air. For embryos and larvae, this involves placing the abalone in seawater-filled containers in a cooler. For juveniles and adults, this involves removing the animals from the substrate and placing them in coolers with moist towels. The animals will be out of the water for several hours (less than 24 hours). Researchers will minimize stress, injury, and mortality to the animals by implementing the best practices described in established transport protocols. These best practices include monitoring and maintaining appropriate temperatures throughout transport.

Researchers have successfully transported adult black abalone between facilities using established methods. Twelve black abalone were successfully transferred from UCSB to the Navy's SSC Pacific facility in 2013 and then to the SWFSC in 2014. Two died while at the SSC Pacific facility (Lapota 2015) and two died at the SWFSC La Jolla lab (pers. comm. with Paula Sylvia, SWFSC, on 4 April 2015), most likely due to health issues rather than the effects of transport. In 2016, an additional eight black abalone were transferred from UCSB to the SWFSC Lab with no mortalities. In 2017, nine black abalone were collected from a field site as part of an emergency rescue response effort and transported to the SWFSC Lab; three died due to injuries sustained during collection.

Researchers have also successfully transported abalone eggs, larvae, and juveniles using established methods. Researchers within the white abalone captive breeding program (covered under Permit 14344-2R) routinely transport fertilized eggs, larvae, and juveniles between facilities by vehicle and by air. Injuries and mortality associated with transport activities appear to be low. For example, in 2015, researchers transported 200 juvenile white abalone in coolers by vehicle from BML to several southern California facilities, with only two mortalities reported within two months of transport (pers. comm. with Kristin Aquilino, BML, on 8 Sept. 2015; with John Hyde, SWFSC, on 29 Dec. 2015; and with Kiersten Darrow, Cabrillo Marine Aquarium, on 3 Jan. 2016).

We expect abalone to experience minor, temporary stress from transport activities. As stated above, removal from the substrate can cause injuries; however, researchers will implement several measures to minimize stress and injury during removal. Researchers will also implement best practices to minimize stress during handling and transport, such as monitoring and maintaining appropriate moisture, air, and temperature levels throughout transport. We expect injuries and mortality associated with transport activities to be limited to a few individuals.

2.5.1.3. Tagging

Permit 19571-2R would allow researchers to tag black abalone by attaching a small numbered tag or PIT tag to the shell using glue or marine epoxy. Tagging should only need to be conducted once, unless the tag falls off. Shell tagging methods have been used in the lab and in the field with minimal effects on abalone (Hale et al. 2012; Richards and Whitaker 2012).

We expect tagging to cause minor, temporary stress to individual abalone due to being handled and kept out of the water for a short time. To minimize stress, researchers will keep the animals moist by placing them on a wet towel and limiting the time out of the water to a few minutes.

2.5.1.4. Health Assessments and Treatments

Permit 19571-2R would allow researchers to visually examine the black abalone to assess their health and apply treatments as needed. Health assessment and treatment activities include: (1) OTC treatment to remove the withering syndrome pathogen; (2) sabellid worm inspections; and (3) wax treatments to remove sabellid worms and other shell-boring organisms.

Disease outbreaks are a concern for black abalone in captivity. Researchers have implemented several measures to minimize the risk of withering syndrome. These measures include quarantining new abalone upon arrival at a facility, OTC treatment to eliminate the withering syndrome pathogen from infected animals, UV treatment of incoming seawater (to kill the pathogen), regular fecal sampling and testing, and maintaining cool water temperatures. Collection of fecal swab samples may cause minor, short-term stress. The OTC bath treatment (Moore 2015) involves immersing the abalone in an oxytetracycline solution for 24 hours at a time, for eight times in total. Abalone will experience stress during this procedure, because the animals are exposed to an antibiotic solution and are not fed for two periods spanning several days. The stress may cause animals to release or re-absorb any gametes, disrupting their reproductive development. This bath treatment has been applied successfully at several facilities throughout California without killing abalone or causing long-term harm. Abalone may experience short-term stress, but we do not expect the procedure to kill or cause long-term harm to individuals. The treatment will also remove the pathogen and minimize the likelihood that the abalone will develop withering syndrome and die.

Sabellid-worm inspections will be conducted on all new abalone brought into facilities, as well as once per year at facilities certified to be sabellid-free. Inspections may involve keeping the abalone out of the water for up to 30 minutes. In most cases the abalone will only be out of the water for a few minutes. Because black abalone are intertidal and can remain out of water for several hours, we do not expect the abalone to experience more than minor, temporary stress due to being handled and kept out of water for a short time. To minimize stress, researchers will keep the animals moist by placing them on wet towels.

Shell waxing will be conducted to remove sabellid worms and infestations of boring organisms on the abalone shells. We expect abalone to experience minor, temporary stress from handling during the shell waxing procedure. Researchers will minimize stress by only conducting shell waxing when necessary to prevent damage to the shell (e.g., when there is evidence of live *Polydora* covering the shell, or more than 50 percent of the surface of the shell shows evidence of boring organisms). Researchers will also keep the abalone moist, avoid covering the respiratory pores, and limit the time out of the water (e.g., the procedure should take less than 10 minutes). Researchers have successfully conducted the shell waxing protocol on black abalone (and other abalone species), with minimal effects on the abalone (SWFSC 2021).

2.5.1.5. Genetic Sampling

Permit 19571-2R would allow researchers to collect swab samples or tissue samples for genetic analysis. To collect swab samples, researchers will use a buccal swab to swab the soft tissue or shell of the abalone. To collect epipodial clippings, researchers will clip the end of one of the epipodial tentacles on the sides or posterior of the abalone.

We expect swab sampling to cause minor, temporary stress to individuals, with little to no long-term effects. Touching the animals may cause them to clamp down more tightly onto the substrate; in rare cases, abalone may move. Tissue sampling using the epipodial clipping method would injure the abalone, but we expect injuries to be minor and unlikely to cause long-term harm or injury to the animals. The epipodial clipping method is a well-established, non-lethal method that has been used to collect tissue samples from abalone in the field and in captivity, with minimal effects on individuals (Hamm and Burton 2000; Gruenthal and Burton 2005; Gruenthal et al. 2014; Coates et al. 2014).

2.5.1.6. Collection of Dead or Obviously Sick/dying Abalone

Permit 19571-2R would allow researchers to preserve dead black abalone, as well as intentionally kill and preserve obviously sick/dying animals, thus killing the animals before they die naturally. This is necessary to preserve the tissues for analysis, to determine the cause of death. To reduce the likelihood that a healthy abalone may be collected and killed, researchers will only sacrifice abalone that are identified as obviously sick/dying based on the following symptoms: reduction or cessation of feeding, extreme lethargy, withered and discolored foot muscle, and/or inability to adhere to the substrate. Abalone showing these symptoms are expected to die within a few days. Specimens, samples, and/or parts will be frozen or preserved and sent to labs for necropsy and analysis. Removal of dead and obviously sick/dying abalone may reduce the spread of pathogens to other abalone within the facility.

2.5.1.7. Broodstock Conditioning Studies

Permit 19571-2R would allow researchers to conduct broodstock conditioning studies to identify factors to improve reproductive condition in adult black abalone. Researchers will evaluate the following factors: diet, water temperature, seasonal photoperiod, tidal cycles, and reproductive hormones. Researchers will expose broodstock to different conditions and regularly assess their reproductive condition by measuring their weight, shell length, and gonad index.

These studies will involve removing the abalone from the tanks no more than once per month, except during hormone injection experiments which may require more regular handling (weekly over eight weeks). We expect black abalone to experience minor, temporary stress in response to removal and handling and exposure to varying conditions. Removal may cause minor cuts to the foot muscle. We do not expect hormone injections to cause injuries to abalone, based on the permit applicant's trials involving red abalone that did not result in any injuries or mortality (see permit application).

Researchers will minimize stress and injury by carefully removing abalone from the tanks by hand or by using a flat instrument (e.g., a plastic spatula and an abalone iron). Researchers will

limit the handling time and time out of the water. Researchers will also keep experimental conditions within the species' known natural range for temperature, photoperiod, and tidal cycles, as well as use macroalgae known to be encountered by black abalone in the wild. Studies will be short in duration (1-3 months). Individual abalone will not be subjected to more than two experimental trials per year.

2.5.1.8. Spawning Induction Studies

Permit 19571-2R would allow researchers to conduct spawning studies using standard and modified methods to induce spawning. Standard methods include desiccation, thermal treatments, and hydrogen peroxide treatments. Modified methods include the use of extended desiccation periods, seasonal tidal cycles, and hormones.

We expect spawning activities to cause temporary stress to black abalone due to removal from the substrate, handling, and exposure to varying conditions. Removing animals from the substrate may cause minor cuts to the foot muscle. We do not expect hormone injections to cause injuries to the abalone, based on the permit applicant's trials involving red abalone that did not result in any injuries or mortality (see permit application).

Throughout the spawning experiments, researchers will closely monitor the abalone for signs of stress. Researchers will minimize stress and injury to individual black abalone by carefully removing abalone by hand or using a flat instrument. Researchers will also minimize handling time and maintain water circulation and proper holding conditions throughout the trials. Researchers will not expose the abalone to conditions outside their natural range for water temperatures, desiccation periods, and tidal cycles. Experienced researchers will conduct the spawning trials. A maximum of four spawning trials will be conducted per year and individual abalone will not be subject to more than two experimental trials per year. If spawning is successful, researchers will follow well-established methods for larval rearing and settlement and juvenile grow-out.

2.5.1.9. Juvenile Growth and Survival Studies

Permit 19571-2R would allow researchers to evaluate different temperatures and diets to optimize the growth of captive-bred juveniles. These studies will involve removing and handling the juveniles on a regular (e.g., monthly) basis to assess their growth.

We expect black abalone to experience minor, temporary stress in response to removal, handling, and exposure to varying conditions. Removal may cause minor cuts to the foot muscle. Researchers will minimize stress and injury by carefully removing abalone from the tanks by hand or using a flat instrument. Researchers will limit the handling time and time out of the water. Temperature studies will be short in duration (3-6 months) and juveniles will not be exposed to temperatures beyond their normal range. Diet studies will only include macroalgal species that juveniles would encounter in the wild or artificial manufactured feed that is known to be safe for abalone.

2.5.1.10. Population and Species Level Effects

We evaluate how the effects of the proposed permit at the individual level may affect black abalone at the population and species level. We conclude by evaluating whether the proposed activities could appreciably reduce the species' likelihood of surviving and recovering in the wild. We consider the effects of the proposed permit within the context of the species' status and recovery needs.

Permit 19571-2R would allow the approved facilities to maintain the current captive population of black abalone, as well as receive additional black abalone from other sources. We do not expect that receiving, maintaining, and conducting captive research activities on these black abalone will reduce the species' survival and recovery in the wild. These abalone were already removed from the wild prior to the ESA-listing or will have been removed for other reasons (e.g., due to poaching or in response to an emergency event). Permit 19571-2R would simply allow these abalone to be incorporated into ongoing research and enhancement activities, to benefit the captive program by increasing the abundance and genetic diversity of the captive population.

We expect most of the proposed activities under Permit 19571-2R to cause minor, sublethal effects on individual black abalone, with little to no long-term effects. Sublethal effects include mild stress and minor injuries (e.g., minor scrapes or cuts to the foot muscle). These sublethal effects may cause minor, temporary reductions in growth and development at the individual level. Researchers will implement measures to minimize stress and injury. We do not expect the stress, injuries, and minor, temporary reductions in growth and development at the individual level to affect the viability of the captive population.

We do not expect the proposed permit activities to kill abalone, except for: (a) collection of a small sample of individuals to document survival and development of early life stages; and (b) sacrifice of obviously sick/dying abalone for necropsy. Researchers plan to collect a small number of captive-bred individuals to evaluate survival and document early life development, including eggs, larvae, and newly settled juveniles up to a few months in age. The sample collected will be a very small proportion of the number produced (millions of eggs and larvae; thousands of juveniles) and very small compared to the number expected to die of natural mortality (natural mortality is 99.5 to 100 percent for the larval to one-year old juvenile stage, based on captive-raised white abalone; unpublished data by Kristin Aquilino, BML, on 20 January 2016). We do not expect this collection to reduce the viability of the captive population. When necessary, researchers will sacrifice obviously sick/dying abalone for necropsy. Obviously sick/dying abalone are expected to die within a few days. Sacrificing these abalone would preserve their tissues for analysis so that researchers can assess the potential cause of death and improve health monitoring and maintenance for the captive population.

We expect natural mortality across all life stages, particularly in the early life stages (embryo to six-month old) when mortality rates can be nearly 100 percent. We recognize the possibility that all of the captive abalone could die over the life of the permit, due to natural mortality or catastrophic events; however, the likelihood of this happening is low for several reasons. First, researchers will implement best practices for abalone health and husbandry, including disease and parasite management protocols to minimize disease outbreaks. Second, as part of the

proposed permit activities, researchers will develop optimal conditions for juvenile survival and growth. Third, the proposed permit would require researchers to notify NMFS of any unusual mortality events, providing an opportunity to review the facility's operations and implement improvements to optimize the health and survival of the captive population. Finally, adult black abalone are currently held at multiple facilities throughout the coast, providing some protection to the captive population should an unusual mortality event occur at one facility.

In summary, we expect the proposed permit activities to result in minor stress and injuries to black abalone. Generally, we expect individual abalone to recover quickly from stress and injuries. At most, individual abalone may experience short-term reductions in growth and development. Though possible, we do not expect all of the captive abalone to die over the duration of the five-year permit; however, we do expect some portion to die due to natural causes and a small number of captive-bred individuals to be killed and preserved to document survival and development of early life stages. We expect the proposed permit conditions to sufficiently minimize stress, injuries, and mortality in the captive population. Maintaining a healthy captive population and developing reliable spawning and culturing methods will ultimately benefit species recovery in the wild. The ability to captively propagate and grow-out black abalone will provide valuable information on the species' biology and life history to inform field monitoring, management, and recovery efforts. Captive-bred abalone may also be used in laboratory studies and in field planting efforts to enhance wild populations.

2.6. Cumulative Effects

"Cumulative effects" are those effects of future state or private activities, not involving Federal activities, that are reasonably certain to occur within the action area of the Federal action subject to consultation (50 CFR 402.02 and 402.17(a)). Future Federal actions that are unrelated to the proposed action are not considered in this section because they require separate consultation pursuant to section 7 of the ESA.

Some continuing non-Federal activities are reasonably certain to contribute to climate effects within the action area. However, it is difficult, if not impossible, to distinguish between the action area's future environmental conditions caused by global climate change that are properly part of the environmental baseline *vs.* cumulative effects. Therefore, all relevant future climate-related environmental conditions in the action area are described in the environmental baseline (Section 2.4).

We expect the threats and factors described in Section 2.4 (Environmental Baseline) will continue to affect black abalone populations in captivity. For example, withering syndrome will continue to pose a threat to captive populations. We expect this threat to decline or remain stable into the future, given implementation of health monitoring and treatment protocols by researchers.

As discussed in Section 2.4 (Environmental Baseline), an unprecedented red tide event in 2020 resulted in poor water quality that killed one black abalone at the SWFSC La Jolla Lab (SWFSC 2021). Multiple factors may influence the occurrence, severity, and frequency of red tides and other harmful algal blooms (HABs), including nutrients from upwelling, river runoff, and wastewater discharge (Howard et al. 2014), as well as climate effects (Nezlin et al. 2018).

Additional HABs may occur during the permit period and affect water quality conditions and the captive black abalone population.

In response to the coronavirus/COVID-19 pandemic, California implemented state-wide restrictions that affected researchers' ability to carry out research activities under Permit 19571 in 2020 through the present. For example, facilities were required to limit personnel, access, and activities to focus on what was essential to keep the captive animals alive. Researchers were not able to carry out broodstock conditioning or spawning activities. It is uncertain when facilities will be allowed to operate at full capacity.

NMFS issued a final recovery plan for black abalone in November 2020 to guide recovery activities (NMFS 2020). Recovery actions identified in the plan include long-term population monitoring, population and habitat restoration where populations have declined, disease research and management plans, emergency response plans, coordination with Mexico, and outreach and education. Implementation of this recovery plan is expected to increase collaboration and on-the-ground recovery efforts for black abalone.

Other non-Federal activities that could affect captive black abalone populations include changes to State regulations and requirements for captive holding facilities and for the import of invertebrates, which can affect the introduction and spread of abalone diseases. We visited CDFW's webpages for Marine Aquaculture (https://www.wildlife.ca.gov/Conservation/Marine/ABMP/Aquaculture) and the Shellfish Health Laboratory (https://www.wildlife.ca.gov/Conservation/Laboratories/Shellfish-Health). We did not find any information indicating changes in State regulations or requirements that may affect captive facilities or the introduction and spread of abalone diseases.

We did not identify additional state or private activities that are reasonably certain to occur within the action area and that could result in cumulative effects on black abalone. Introduction of other pathogens and parasites could occur within the time frame of the permit and affect both wild and captive populations. However, the potential effects are difficult to evaluate at this time, given the unpredictability and uncertainty in the timing, location, scope, and severity of such events. Although we have examples of how other pathogens have affected abalone in other parts of the world, there are many uncertainties regarding whether and when these pathogens could spread to California and the effects on black abalone versus other abalone species. To date, no outbreaks of these other pathogens have been observed in wild or captive black abalone populations in California.

2.7. Integration and Synthesis

The Integration and Synthesis section is the final step in our assessment of the risk posed to species as a result of implementing the proposed action. In this section, we add the effects of the action (Section 2.5) to the environmental baseline (Section 2.4) and the cumulative effects (Section 2.6), taking into account the status of the species (Section 2.2), to formulate the agency's biological opinion as to whether the proposed action is likely to reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing its numbers, reproduction, or distribution.

2.7.1. Black Abalone

Black abalone populations have declined throughout a large portion of their range. Although historical overfishing contributed to these declines, the main cause was disease-induced mass mortalities. Affected populations remain at low abundance and density and are subject to demographic risks such as reduced reproduction and recruitment, loss of genetic diversity, and poor connectivity among populations. Recruitment has been observed at a few sites in southern California, indicating some natural recovery is occurring. Populations north of Cayucos remain healthy and stable, but withering syndrome may spread northward with warm water events. Other threats, such as illegal harvest and elevated water temperatures, continue to affect black abalone populations. Oil spills, sedimentation events, and other pathogens also pose a potential threat to the species. Threats of sedimentation and illegal harvest may increase in the coming years, given the severe wildfires and increases in intertidal harvest and human use activities observed in 2020-2021. At the same time, conservation efforts are ongoing to enforce protections, advance our understanding and management of threats, improve habitat, and increase public awareness of abalone conservation needs. Continued long-term monitoring is critical to track the species' status and progress toward recovery; research and enhancement studies are important to advance recovery tools.

The proposed permit activities would directly address species recovery needs. The Final Black Abalone Recovery Plan (NMFS 2020) identifies captive propagation methods as an important tool for species recovery. The ability to captively propagate black abalone would provide animals for critical research on the species' biology, life history, development, and response to threats. For example, captive-bred abalone may be used to evaluate the effects of different types of oil on larval and juvenile black abalone (note that such studies would need to be authorized under an ESA permit; the effects of such studies are not included or evaluated in this opinion). This information would inform our response to oil spills. Captive-bred black abalone may also be used to re-establish and enhance wild populations through field planting efforts.

The proposed permit activities would involve take of wild-origin black abalone that are currently held in captivity or that may be brought into captivity as a result of law enforcement cases, emergency response activities, or Federal actions. The proposed activities would also involve take of captive-bred black abalone. Although the proposed permit would authorize extensive take of black abalone, we expect the effects to primarily be limited to minor, short-term stress and minor injuries to individuals. At most, these effects could result in short-term reductions in the growth or development of individuals; however, we expect individuals to recover quickly with little to no effect on long-term growth, survival, and reproductive development. Researchers will intentionally kill a small number of captive-bred abalone to estimate survival and document early life development, representing a very small proportion of the captive-bred progeny. We expect losses due to natural mortality across all life stages, ranging from very high during the first year (up to 100 percent mortality from the embryo to one-year old stage) to very low (about five percent per year) from the one-year old to adult stages. Researchers will implement measures to minimize stress, injury, and mortality. These include maintaining optimal holding conditions, limiting removal and handling to the minimum necessary, and implementing best practices for animal health and husbandry, such as routine health assessments and treatments to remove pathogens and parasites as needed. We believe these measures sufficiently minimize the potential effects of the proposed permit activities on the captive population.

Considering the status of the species, the environmental baseline, and cumulative effects, we do not expect the proposed permit activities to reduce fitness at the population or species level. The proposed permit activities would support and enhance recovery efforts by developing reliable methods to captively propagate black abalone and produce healthy black abalone for use in critical research studies and population restoration efforts. Overall, the proposed permit activities would contribute to effective management and recovery strategies for the species.

2.8. Conclusion

After reviewing and analyzing the current status of the listed species, the environmental baseline within the action area, the effects of the proposed action, the effects of other activities caused by the proposed action, and cumulative effects, it is NMFS' biological opinion that the proposed action is not likely to jeopardize the continued existence of endangered black abalone. Critical habitat has been designated for black abalone but does not occur within the action area; therefore, effects on designated black abalone critical habitat were not analyzed.

2.9. Incidental Take Statement

Section 9 of the ESA and Federal regulations pursuant to section 4(d) of the ESA prohibit the take of endangered and threatened species, respectively, without a special exemption. "Take" is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct. "Harm" is further defined by regulation to include significant habitat modification or degradation that actually kills or injures fish or wildlife by significantly impairing essential behavioral patterns, including breeding, spawning, rearing, migrating, feeding, or sheltering (50 CFR 222.102). "Incidental take" is defined by regulation as takings that result from, but are not the purpose of, carrying out an otherwise lawful activity conducted by the Federal agency or applicant (50 CFR 402.02). Section 7(b)(4) and section 7(o)(2) provide that taking that is incidental to an otherwise lawful agency action is not considered to be prohibited taking under the ESA if that action is performed in compliance with the terms and conditions of this ITS.

For the action considered in this opinion, there is no incidental take. The reason for this is that all the take contemplated in this opinion would be carried out under a permit that allows the permit holder to directly take black abalone. The actions are considered to be direct take rather than incidental take, because in every case their actual purpose is to take the animals as part of a lawfully permitted activity. Thus, the take cannot be considered "incidental" under the definition given above. Nonetheless, one of the purposes of an incidental take statement is to lay out the amount or extent of take beyond which individuals carrying out an action cannot go without being in possible violation of section 9 of the ESA. That purpose is fulfilled here by the amounts of direct take laid out in the effects section above (Table 1 in Section 2.5.1). Those amounts constitute hard limits on both the amount and extent of take the permit holders would be allowed in a given year. This concept is also reflected in the reinitiation clause below.

2.10. Conservation Recommendations

Section 7(a)(1) of the ESA directs Federal agencies to use their authorities to further the purposes of the ESA by carrying out conservation programs for the benefit of threatened and endangered species. Specifically, conservation recommendations are suggestions regarding discretionary measures to minimize or avoid adverse effects of a proposed action on listed species or critical habitat or regarding the development of information (50 CFR 402.02).

NMFS has the following conservation recommendations:

(1) The permit holder and researchers under the permit should consider developing a central repository for biological samples collected and analyzed. The specimens in the repository should be linked to a central database providing metadata on the specimens.

(2) The permit holder and researchers under the permit should consider developing a forum for sharing data (e.g., results of experimental research studies) and public outreach and education materials with one another, to inform all the project partners.

We request that the Permit Holder notify NMFS regarding implementation of these conservation recommendations. The proposed permit will require the Permit Holder to summarize their progress on these conservation measures in the annual reports.

2.11. Reinitiation of Consultation

This concludes formal consultation for the NMFS WCR Permit Team's proposal to issue Permit 19571-2R to the SWFSC to take black abalone for research and enhancement purposes pursuant to the provisions of Section 10(a)(1)(A) of the ESA.

As 50 CFR 402.16 states, reinitiation of consultation is required and shall be requested by the Federal agency or by the Service where discretionary Federal agency involvement or control over the action has been retained or is authorized by law and if: (1) The amount or extent of incidental taking specified in the ITS is exceeded, (2) new information reveals effects of the agency action that may affect listed species or critical habitat in a manner or to an extent not considered in this opinion, (3) the identified action is subsequently modified in a manner that causes an effect to the listed species or critical habitat that was not considered in the biological opinion, or (4) a new species is listed or critical habitat designated that may be affected by the action.

In the context of this opinion, there is no incidental take anticipated and the reinitiation trigger set out in (1) is not applicable. If any of the direct take amounts specified in this opinion's effects analysis in Section 2.5 are exceeded, reinitiation of formal consultation will be required because the regulatory reinitiation triggers set out in (2) and/or (3) will have been met.

2.12. "Not Likely to Adversely Affect" Determinations

NMFS does not anticipate the proposed action will adversely affect endangered white abalone. The proposed permit activities will occur at approved facilities that hold both black abalone and

white abalone. Captive holding of black abalone at these facilities may increase the risk of disease and parasite infections for white abalone, which are also susceptible to withering syndrome, sabellid worms, and other shell-boring organisms. To minimize effects on captive white abalone at the facilities, researchers will quarantine newly acquired black abalone and treat the animals for disease if needed. Researchers will also hold black abalone in separate tanks and systems from other abalone at the facilities. The proposed permit activities will only involve black abalone and will not involve white abalone at the facilities.

We expect that these measures sufficiently minimize the potential risk to white abalone and other captive abalone species held at the approved facilities. The potential for the proposed permit activities to increase the risk of disease and parasite infections for white abalone is extremely unlikely and discountable. Therefore, we conclude that the proposed permit activities are not likely to adversely affect white abalone.

3. DATA QUALITY ACT DOCUMENTATION AND PRE-DISSEMINATION REVIEW

The Data Quality Act (DQA) specifies three components contributing to the quality of a document. They are utility, integrity, and objectivity. This section of the opinion addresses these DQA components, documents compliance with the DQA, and certifies that this opinion has undergone pre-dissemination review.

3.1. Utility

Utility principally refers to ensuring that the information contained in this consultation is helpful, serviceable, and beneficial to the intended users. The intended user of this opinion is the NMFS WCR Permits Team. Other interested users could include the Permit Holder and Principal Investigator (SWFSC, John Hyde), co-investigators listed on the permit application, and abalone researchers. Individual copies of this opinion were provided to the NMFS WCR Permits Team. The document will be available within two weeks at the NOAA Library Institutional Repository [https://repository.library.noaa.gov/welcome]. The format and naming adheres to conventional standards for style.

3.2. Integrity

This consultation was completed on a computer system managed by NMFS in accordance with relevant information technology security policies and standards set out in Appendix III, 'Security of Automated Information Resources,' Office of Management and Budget Circular A-130; the Computer Security Act; and the Government Information Security Reform Act.

3.3. Objectivity

Information Product Category: Natural Resource Plan

Standards: This consultation and supporting documents are clear, concise, complete, and unbiased; and were developed using commonly accepted scientific research methods. They adhere to published standards including the NMFS ESA Consultation Handbook, ESA regulations, 50 CFR 402.01 et seq., and the MSA implementing regulations regarding EFH, 50 CFR 600.

Best Available Information: This consultation and supporting documents use the best available information, as referenced in the References section. The analyses in this opinion contain more background on information sources and quality.

Referencing: All supporting materials, information, data and analyses are properly referenced, consistent with standard scientific referencing style.

Review Process: This consultation was drafted by NMFS staff with training in ESA, and reviewed in accordance with West Coast Region ESA quality control and assurance processes.

4. REFERENCES

4.1. Personal Communications and Unpublished Data

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