Papahānaumokuākea Marine National Monument

RESEARCH Permit Application

NOTE: ***This Permit Application (and associated Instructions) are to propose activities to be conducted in the Papahānaumokuākea Marine National Monument. The Co-Trustees are required to determine that issuing the requested permit is compatible with the findings of Presidential Proclamation 8031. Within this Application, provide all information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historic, and cultural resources of the Papahānaumokuākea Marine National Monument (Monument).***

ADDITIONAL IMPORTANT INFORMATION:

- Any or all of the information within this application may be posted to the Monument website informing the public on projects proposed to occur in the Monument.

- In addition to the permit application, the Applicant must either download the Monument Compliance Information Sheet from the Monument website OR request a hard copy from the Monument Permit Coordinator (contact information below). The Monument Compliance Information Sheet must be submitted to the Monument Permit Coordinator after initial application consultation.

- Issuance of a Monument permit is dependent upon the completion and review of the application and Compliance Information Sheet.

INCOMPLETE APPLICATIONS WILL NOT BE CONSIDERED

Send Permit Applications to:
Papahānaumokuākea Marine National Monument Permit Coordinator
6600 Kalaniana'ole Hwy. # 300
Honolulu, HI 96825
nwhipermit@noaa.gov
PHONE: (808) 397-2660 FAX: (808) 397-2662

SUBMITTAL VIA ELECTRONIC MAIL IS PREFERRED BUT NOT REQUIRED. FOR ADDITIONAL SUBMITTAL INSTRUCTIONS, SEE THE LAST PAGE.

Papahānaumokuākea Marine National Monument
Permit Application Cover Sheet

This Permit Application Cover Sheet is intended to provide summary information and status to the public on permit applications for activities proposed to be conducted in the Papahānaumokuākea Marine National Monument. While a permit application has been received, it has not been fully reviewed nor approved by the Monument Management Board to date. The Monument permit process also ensures that all environmental reviews are conducted prior to the issuance of a Monument permit.

Summary Information

Applicant Name: Christopher E. Bird & Robert J. Toonen
Affiliation:
[1]Department of Life Sciences, Texas A&M University – Corpus Christi
[2]Hawai‘i Institute of Marine Biology, University of Hawai‘i at Mānoa

Permit Category: Research
Proposed Activity Dates: June-July 2014, targeting June 19-July2
Proposed Method of Entry (Vessel/Plane): Vessel
Proposed Locations: Intertidal and shallow water habitats around basaltic islands on which 'opihi occur. Specifically, Nihoa Island, Mokumanamana Island, Mokupapapa (French Frigate Shoals, La Perouse Pinnacles,), and Puhahonu (Gardner Pinnacles)

Estimated number of individuals (including Applicant) to be covered under this permit: Eleven total people will be covered under this permit, co-listed under the Native Hawaiian Practices application submitted by Shauna Kehaunani Springer.
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Estimated number of days in the Monument: approximately 12 days

Description of proposed activities: (complete these sentences):
a.) The proposed activity would…
aim to examine the biodiversity of the Hawaiian intertidal and shallow subtidal ecosystem, and study the basic ecology of 'opihi populations within the NWHI. We propose to continue conducting the first comprehensive biodiversity mapping survey of the intertidal zone in the NWHI and quantify species presence/absence and relative abundances within and among sites across the basaltic emergent islands. We also seek to examine population connectivity of intertidal species in comparison to the broad survey of coral reef organisms sampled to date. We find different patterns of larval exchange among the ‘opihi which suggests that intertidal species may differ from the average seen in subtidal taxa, and that has important management implications that need to be confirmed. We propose to examine the reproductive status ‘opihi populations across the NWHI to better understand natural population dynamics and potential mechanisms of speciation in these economically, ecologically and culturally important limpets.

This work will be tightly linked with the Native Hawaiian cultural practice application and is a joint collaborative study among Na Mamo o Muole'a, the Nature Conservancy, the Hawai‘i Institute of Marine Biology, Nā Maka o Papahānaumokuākea, and the NOAA Papahānaumokuākea Marine National Monument. We will perform the standardized 'opihi monitoring protocol developed through this collaboration, which is inclusive of Hawaiian methods of monitoring, has was specifically developed (and is continuously being refined) to monitor intertidal populations associated with ‘opihi across the Main and Northwestern Hawaiian Islands. To date, communities on every island, save Ni'ihau, have been involved and through these efforts the NWHI have been surveyed for intertidal species composition, population size and age structure of organisms associated with ‘opihi. Here we request a permit to conduct the sixth year of surveys and monitoring within the NWHI, with a primary focus on mapping opihi population sizes.

b.) To accomplish this activity we would ….
conduct standardized transect and rapid mapping surveys developed collaboratively among the partners listed above to integrate quantitative scientific data collection with Native Hawaiian observational data. Specifically, we will lay a minimum of 15 belt transects per island to assess size distribution, population density, community structure, species range, distribution, and rugosity for all identifiable organisms within the intertidal zone. Rapid mapping surveys will be conducted where the number of opihi (separate counts for *Cellana exarata* and *Cellana sandwicensis*) and presence/absence for other invert species are recorded in two meter wide belt transects at 10s-100s of georeferenced points around each island. Using this method, we were able to census all ‘opihi residing on Mokupapapa in 2013.

We will collect ‘opihi and a handful of very common intertidal species to examine reproductive state and patterns of population connectivity in the intertidal zone and compare that directly to the patterns found in subtidal species. The size and state of ‘opihi gonads will be determined in the laboratory after the cruise. Genomic DNA isolated from inverteberate muscle tissues will be sequenced in order to assess connectivity and stock structure. Messenger RNA, the products of gene expression, will be isolated from gonad tissue in order to identify and compare the ‘opihi sperm-egg recognition proteins (methods described below), and in the accompanying Native Hawaiian Practices Permit Application filed by Kehau Springer. When the ship leaves the island, no supplies will be left behind. The samples we request to be collected for this work are summarized in Appendix 1. All data will be stored and analyzed at Texas A&M University Corpus Christi and the Hawaii Institute of Marine Biology by Chris Bird and Rob Toonen, respectively. Tissue samples, DNA and RNA sampled from animals will need to be additionally process at specialized laboratories at Texas A&M University – College Station, ARQ Genomics in Austin, TX, and Simon Frasier University in Vancouver, BC. These data will be useful to both the Monument, as well as to local and governmental resource managers in the Main Hawaiian Islands to make effective decisions on managing the resources.

c.) This activity would help the Monument by …

providing baseline knowledge of one of the least studied ecosystems which is potentially most threatened by climate change . Sea level rise is underway, and the first community to feel the effects of climate change will be the one that lives at the interface of land and sea and experiences the greatest extremes of both environments: the intertidal. Limited knowledge of this ecosystem restricts our understanding of climate change impacts and suitable responses. Further, knowing which species occur and where they live is fundamental to the management of natural resources in any ecosystem, and the Hawaiian intertidal zone is poorly characterized in general. We will also confirm whether or not the intertidal species show a distinct pattern of population connectivity across the archipelago than do the subtidal ones surveyed to date. These data will provide quantitative data on the species present in these ecosystems, their biodiversity, population dynamics and connectivity and also contribute to the ongoing debate about how new species arise in the sea. The tight collaboration of the team comprised of cultural practitioners, research scientists, and resource managers will ensure that the findings are of relevance to a broad group of stakeholders and of direct relevance to the people of Hawai‘i.

Other information or background: Littoral habitats, those lying between the low-tide line and the upper limit of aquatic species on the shore, are among the most studied and well-known aquatic habitats on the planet. A primary exception to that generalization is that this zone is one of the least studied in Hawai‘i despite six consecutive years of surveying in the Hawaiian Islands by members of the ‘Opihi Partnership. The effects of tides on littoral marine habitats are so ubiquitous that shorelines are commonly described as ‘intertidal’, whereas waves are considered a secondary factor that simply modifies the intertidal habitat. However in Hawai‘i, mean significant wave height exceeds tidal range most of the time, and may be a primary structuring force for littoral communities as outlined in Bird (2006) and Bird et al (2013). The patterns of distribution and abundance of organisms on rocky shores, in particular the upper and lower limits of species, along vertical gradients of exposure have been studied extensively in other regions of the globe. Hypotheses addressing the causes of biotic zonation and community structure have evolved from strictly physical to an inseparable combination of physical and biological factors, including physiological tolerance (Connell 1961a b), species interactions (Bruno & Bertness 2001, Menge & Branch 2001), and all other forms of biotic factors.

A fundamental advance in the understanding of biotic zonation on rocky shores was the demonstration that species interactions also affected zonation patterns, where biotic factors generally affect the lower limit of distribution and physical factors affect the upper limit of distribution (Connell 1961a b, Paine 1967). A number of exceptions to this generalization have been demonstrated, many of which highlight the more general effect of biological interactions on the realized distribution of a species. Ultimately, the inseparable interaction between physical and biological factors define the realized limits of species (Denny & Wethey 2001), and intertidal communities are unique in that organisms must cope with some of the most severe extremes of both marine and terrestrial environments. This has led to debate about whether these species are so hardy that they are resistant to change, or whether they live in such extreme environments that climate change will impact them more (e.g., Stillman 2003). Available data from long-term surveys of the intertidal community in California suggest the latter: intertidal communities are one of the first to show ecosystem impacts of climate change that can already

be documented and are expected to accelerate given future climate change scenarios (e.g., Barry et al 1995; Sagarin et al. 1999).

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial):

Bird, Christopher E.
Assistant Professor, Texas A&M University - Corpus Christi

Toonen, Robert J.
Research Professor, HIMB, University of Hawai'i at Mānoa

1a. Intended field Principal Investigator (See instructions for more information):
Chris Bird

2. Mailing address (street/P.O. box, city, state, country, zip):

Chris Bird
[redacted]

Rob Toonen
[redacted]

3. Affiliation (institution/agency/organization directly related to the proposed project):
Life Sciences, Texas A&M University - Corpus Christi
HIMB, University of Hawai'i at Mānoa

4. Additional persons to be covered by permit. List all personnel roles and names (if known at time of application) here (e.g. John Doe, Research Diver; Jane Doe, Field Technician):
We expect that the final list of cruise personnel will be available soon, but has not yet been finalized. We seek a crew of 11 people drawn from across the partners listed above and these are

the same participants as those on the Native Hawaiian Practices Permit Application filed by Kehau Springer.
We expect that the scientific crew will likely consist of 3 to 4 members, likely drawn from the following list or their equivalent expertise:

Amended List for June 2014 Cruise Dates

Chris Bird (Ph.D., Asst Professor, TAMUCC),
Rob Toonen (Ph.D., Research Professor, HIMB),
Eric Tong (Ph.D. Candidate , HIMB)
Hoku Johnson (PMNM, Resource manager)
Matt Ramsey (former DAR Resource manager, NOAA Fisheries manager)
Makani Gregg
Bert Hispanola (Cultural Practitioner)
James Hispanola (Cultural Practitioner)
Russell Amimoto (Nature Conservancy)
Nakoa Goo (NOAA)
TBD Kaua'i Cultural Practitioner

Additional crew members will be selected from Native Hawaiian communities as outlined in the Springer permit application and be included here as co-listed permittees for a maximum of 12 people in total.

Section B: Project Information

5a. Project location(s):

		Ocean Based	
☒ Nihoa Island	☒ Land-based	☒ Shallow water	☐ Deep water
☒ Necker Island (Mokumanamana)	☒ Land-based	☒ Shallow water	☐ Deep water
☒ French Frigate Shoals	☒ Land-based	☒ Shallow water	☐ Deep water
☒ Gardner Pinnacles	☒ Land-based	☒ Shallow water	☐ Deep water
☐ Maro Reef			
☐ Laysan Island	☐ Land-based	☐ Shallow water	☐ Deep water
☐ Lisianski Island, Neva Shoal	☐ Land-based	☐ Shallow water	☐ Deep water
☐ Pearl and Hermes Atoll	☐ Land-based	☐ Shallow water	☐ Deep water
☐ Midway Atoll	☐ Land-based	☐ Shallow water	☐ Deep water
☐ Kure Atoll	☐ Land-based	☐ Shallow water	☐ Deep water
☐ Other			

NOTE: There is a fee schedule for people visiting Midway Atoll National Wildlife Refuge via vessel and aircraft.

Location Description:
As outlined above, our survey and collection efforts will be concentrated in the intertidal zone, surrounding each emergent basaltic land mass on which 'opihi occur. Although we include this within the land-based category above, the monitoring team would not access any sites beyond the splash zone on any island.

5b. Check all applicable regulated activities proposed to be conducted in the Monument:

☒ Removing, moving, taking, harvesting, possessing, injuring, disturbing, or damaging any living or nonliving Monument resource
☒ Drilling into, dredging, or otherwise altering the submerged lands other than by anchoring a vessel; or constructing, placing, or abandoning any structure, material, or other matter on the submerged lands
☐ Anchoring a vessel
☐ Deserting a vessel aground, at anchor, or adrift
☒ Discharging or depositing any material or matter into the Monument
☐ Touching coral, living or dead
☐ Possessing fishing gear except when stowed and not available for immediate use during passage without interruption through the Monument
☐ Attracting any living Monument resource
☐ Sustenance fishing (Federal waters only, outside of Special Preservation Areas, Ecological Reserves and Special Management Areas)
☐ Subsistence fishing (State waters only)
☒ Swimming, snorkeling, or closed or open circuit SCUBA diving within any Special Preservation Area or Midway Atoll Special Management Area

6 Purpose/Need/Scope ***State purpose of proposed activities:***
We wish to characterize yearly variation in opihi and other intertidal and shallow subtidal populations in PMNM. Further, new genetic techniques that allow us to more fully sequence the genomes of individuals require more stringent sample preservation protocols than were used in the past collections of specimens from PMNM. Using these newer population genomic techniques, we can ascertain a more highly resolved image of connectivity and self recruitment on the islands of PMNM, that include the assessment of unique selective pressures driving local adaptation on the inhabitants of each island.
The primary objectives of this research expedition are to: (1) collect complementary data on the intertidal ecosystem with a suite of research scientists, cultural practitioners, and resource managers; (2) establish a baseline survey of intertidal ecosystems, specifically focused on 'opihi species associations, relative abundance, reproductive cycles, and identity to better understand the implications and consequences of human activities on these communities; (3) determine the species present to characterize the biodiversity of the Hawaiian intertidal zone and their connectivity to one another across the archipelago; (4) to determine ‘opihi size at reproductive maturity and reproductive characteristics such as gonad index, and molecular composition of sperm-egg recognition proteins in the absence of human predation, (5) begin to explore the genomic signatures of adaptation to human activities and natural processes using ‘opihi as a model system to elucidate the mechanisms by which divergent selection can lead to adaptive radiation of marine species.

Along these same lines we request permission to collect up to 3 voucher specimens of an individual organism that cannot be identified as a known species and/or may represent new geographic records or new species from the taxonomic groups under study as laid forth in the voucher specimen guidelines of the Monument. Voucher specimen(s) would be used for taxonomic study to determine the species identity and would be accessioned in an approved repository such as the Bishop and/or Smithsonian museum permanent collections as recommended.

7. Answer the Findings below by providing information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historic, and cultural resources of the Monument:

The Findings are as follows:

a. How can the activity be conducted with adequate safeguards for the cultural, natural and historic resources and ecological integrity of the Monument?
Most activities in this permit application were previously permitted and have demonstrated no impact on the Monument’s cultural, natural and historic resources. The one minor addition, this year, is that in addition to muscle tissues, we are proposing to preserve gonad tissues for molecular analysis. Our research team consists of conservation biologists who are both teaching and studying the science of how best to manage and conserve biological diversity in the sea. As such, minimizing our impact to the ecosystem we are trying to conserve is naturally and inherently a top priority for any research we conduct, especially within the boundaries of the

Monument. We believe that we have implemented every reasonable safeguard for the natural resources and ecological integrity of the Monument in our research, and we do not conduct research that could have a detectable impact on the ecosystem. We have an established track record of management-relevant research in this area and have not been able to detect any cumulative impacts of scientific collections to date (Selkoe et al. 2009). As outlined in greater detail below, our sample size, choice of species, and methodologies have all been selected to provide robust and scientifically rigorous information to managers with the least possible impact to the natural resources of the Monument. We will adhere to all rules, regulations and best practices established by the co-trustees for the Monument, including all quarantine requirements, wildlife viewing guidelines, and entry/exit notification procedures where applicable.

Additionally, our team has always tried and will continue to ensure that we have minimal impact on the cultural resources of Papahānaumokuākea. We rely on our colleagues who are cultural practitioners to take the lead on proper protocols for our voyage, and these are outlined in detail in the accompanying permit application by Springer. Each member of our team is aware of the unique ecological and cultural status of the Monument, and our on-going collaboration with the cultural practitioners continues to expand our understanding of Hawaiian protocol in conducting research within Papahānaumokuākea Marine National Monument.

In addition to following the lead of our cultural practitioner team-mates, we ask that each researcher take responsibility to prepare an appropriate offering in advance to ensure that they reflect on why they are on this trip, what is the purpose of the trip, and enter the Monument with the proper intent. It is respectful to provide an offering and to not go forth to take from the place with empty hands. However, given concerns regarding transport of materials into the Monument, it is also difficult to present a proper offering in the form of a gift. In previous years, we have used pure rainwater collected by hand to ensure a personal connection with the offering, and we believe that this is the best option for research scientists unfamiliar with the proper cultural protocols. This fresh-caught rainwater can be poured out as a personal offering in return for the privilege of collecting samples in the Monument by each member of our team. In addition we will follow the lead and participate to the best of our ability in protocols undertaken by our cultural colleagues in whatever preparation is appropriate for the voyage.

b. How will the activity be conducted in a manner compatible with the management direction of this proclamation, considering the extent to which the conduct of the activity may diminish or enhance Monument cultural, natural and historic resources, qualities, and ecological integrity, any indirect, secondary, or cumulative effects of the activity, and the duration of such effects?

All activities proposed herein have been permitted previously and furthermore was conducted without detectable impact in the past. Our expansion of the scope of the initial surveys to include biodiversity surveys and connectivity work in the intertidal is similar to the work that we have done previously for subtidal reef-associated organisms, and has been done without detectable cumulative impact to date. Our proposed survey of the reproductive status, spawning timing, and larval behavior of 'opihi is likewise expected to have no detectable impact, but will provide valuable scientific and management information for the entire Hawaiian Archipelago. This type of research is directly mandated by the Proclamation, and is necessary to both maintain

ecosystem integrity and provide for adaptive ecosystem management in the face of natural or anthropogenic disasters and global climate change. As outlined above and below, our activities have no detectable effect to diminish Monument resources, nor have any known indirect, secondary or cumulative effects on the ecosystem or resources therein. Because we are conservation biologists who are concerned about exactly these sort of impacts, we have voluntarily conducted a threat assessment of the activities in the Monument (Selkoe et al. 2008) and compiled a cumulative impact threat map of the Monument (Selkoe et al. 2009) which has been provided to the co-trustees for use in future management decisions.

c. Is there a practicable alternative to conducting the activity within the Monument? If not, explain why your activities must be conducted in the Monument.

We expect it is self-evident that there is no practical alternative to sampling within the Monument when the goal of the research is to understand the baseline ecosystem state of the intertidal populations within the Monument. Likewise, surveys of biodiversity within understudied habitats of the Monument which seek to determine the species present, their abundance and distribution are only possible within the bounds of the region of interest. Finally, these studies will be of both direct benefit to the resource management within the Monument itself, and to the remainder of the Hawaiian Archipelago for ecologically, economically and culturally important species such as 'opihi.

The exceptions that may not seem quite so self-evident is the examination of gonad index and gamete recognition proteins. The reproductive work is needed to examine the baseline state of the populations in the absence of human harvest and will provide important information about spawning capacity, timing, ability of larvae to return to their source island, and adaptation of the gamete recognition system to the high natural densities of ‘opihi in PMNM. We humans use our senses to select our mate, but ‘opihi release their gametes into the water column and proteins coating the sperm and eggs mediate mate choice. If eggs can be permissive or selective to fertilization by sperm. If eggs are too permissive, the are fertilized by multiple sperm and the embryo perishes. If the eggs are too restrictive, then the eggs remain unfertilized. In our most recent surveys, we observe upwards of 100 fold higher ‘opihi densities in PMNM relative to Oahu, and we expect that harvesting pressure will leave a distinct signature in the gamete recognition proteins. Females on Oahu are either mostly permissive or else they do not produce offspring. Gamete recognition proteins are also under strong positive selection and the previously discovered levels of gene flow predict that each island might have particular strains of males and females that are more compatible with each other than individuals from other locations. In 2011 -13 we found differences in the reproductive state of ‘opihi between islands and species that could have important implications for the connectivity of populations among islands. Our surveys of genomic and gamete recognition protein diversity can be used to assess the ability of ‘opihi to adapt to human activities.

d. How does the end value of the activity outweigh its adverse impacts on Monument cultural, natural and historic resources, qualities, and ecological integrity?

Given that we can detect no adverse effects of our activities on the resources of the Monument, we believe that the end value of this research clearly outweighs whatever imperceptible impact exists. We have an established track record of communicating our findings to the resource managers and making sure that all research conducted within the Monument meets the bar of management relevance. The proposed research will provide the first quantitative baseline survey of intertidal ecosystems across the Hawaiian Archipelago and address questions of vulnerability to climate change. Additionally, the reproductive work proposed herein will benefit both population studies and resource management of ‘opihi stocks in Hawai‘i, but also contribute to our understanding of how new species can arise in the sea. Finally, the intertidal zone is a greatly understudied ecosystem that is likely to be one of the most directly and immediately impacted by climate change because it experiences the extremes of both terrestrial and marine environments daily. An understanding of the intertidal communities across this region will identify potentially vulnerable locations and species, and (as outlined above) greatly increase the decision-making capacity of the co-trustees in dealing with the reality of future climate change within the Monument and the Hawaiian Archipelago in general.

e. Explain how the duration of the activity is no longer than necessary to achieve its stated purpose.

The expedition length is determined by limited funding, which makes it shorter than ideal, and is certainly no longer than is necessary to accomplish the research goals outlined in this permit application.

f. Provide information demonstrating that you are qualified to conduct and complete the activity and mitigate any potential impacts resulting from its conduct.

Chris Bird has a PhD in Ecology, Evolution, and Conservation Biology through the Botany Department at the University of Hawaii, was a Post Doctoral Fellow at the Hawaii Institute of Marine Biology from 2007-2012 working on the exact type of work entailed in this application, is an Assistant Professor at Texas A&M and has published ~20 research papers in peer-reviewed journals dealing specifically with the subject of conservation and management of Hawaiian natural resources, rocky shores in particular. Rob Toonen has a PhD in Population Biology, is a Research Professor at HIMB, and has published ~200 research papers in peer-reviewed journals dealing specifically with the subject of conservation and management of Hawaiian natural resources. With Rob Toonen and Celia Smith, Chris has been studying ‘opihi and Hawaiian intertidal communities since 1999, before to the establishment of the Monument. This research has been of considerable interest to both the science and management community of Hawai‘i and has begun to receive international recognition for the insights we are gaining to understand divergent selection leading to speciation in the sea. This on-going project should be well known to the Monument co-trustees, and our research accomplishments are presented in regular meetings with the management community and semi-annual meetings. Our accomplishments and qualifications to perform this research are further documented in the included CVs. We will be responsible for the conduct of the scientific team and work closely with the cultural practitioner team to ensure a successful mission.

g. Provide information demonstrating that you have adequate financial resources available to conduct and complete the activity and mitigate any potential impacts resulting from its conduct.

This mission requires no specific funding beyond the cost of mounting the expedition to complete the research goals. The proposed field activities are funded in full by NOAA for the Papahānaumokuākea Marine National Monument. The data analysis and storage will be supported by the Monument as necessary, the Hawai‘i Institute of Marine Biology, and Texas A&M University. As a new faculty at Texas A&M, Chris Bird’s startup package includes funds for three graduate students, as well as ~$500,000 in equipment and supplies required to conduct research. The success of the unfunded ‘opihi partnership demonstrates our collective commitment to this effort and our ability to complete this sort of work voluntarily even in the absence of funding. We are able to leverage the existence of samples to obtain federal funding from a variety of sources, and have an established track record of doing exactly that. We also have a clearly established track record of completing and publishing the research conducted in the Papahānaumokuākea Marine National Monument on a reasonable time frame, and have every intention to continue that tradition. Finally, we provide regular individual research updates to the management community and will also continue that effort in the future.

h. Explain how your methods and procedures are appropriate to achieve the proposed activity's goals in relation to their impacts to Monument cultural, natural and historic resources, qualities, and ecological integrity.

Our choice of sites are guided by personal safety and natural resource concerns within the Monument, but are constrained by the fact that intertidal communities that support ‘opihi populations are limited to basaltic emergent islands. Minimizing our impact on the natural resources of the Monument is critical to us because they are the focus of the study for purposes of conservation, and we absolutely do not want to detract from that system we are seeking to conserve. The methods and procedures we propose to use are widely accepted and are among the few that directly incorporate Native Hawaiian marine practitioners, resource managers and research scientists in collaborative study that is co-designed and jointly implemented. Our success in obtaining extramural funding, our rate of publication in high quality scientific journals, and the frequency with which those studies are cited all show that the work being performed is accepted, valued and endorsed by the global scientific community. Belt transects do not require any specialized equipment and are simple enough to be employed by community members without scientific training across the inhabited Main eight Hawaiian islands. All our work takes full account the unique value and seeks to minimize any potential for impact to the Monument resources.

i. Has your vessel has been outfitted with a mobile transceiver unit approved by OLE and complies with the requirements of Presidential Proclamation 8031?

We will be chartering the Searcher and the partner NOAA PMNM staff will ensure it meets the VMS type-approval requirement as stated in Monument regulations.

j. Demonstrate that there are no other factors that would make the issuance of a permit for the activity inappropriate.

There are no other factors that would make the issuance of the permit inappropriate. The activity is non-commercial. The end-value of the activity is informational and is intended solely to provide local and governmental managers with information critical to the conservation of natural resources.

8. Procedures/Methods:

The primary objectives of this research expedition are to: (1) collect complementary data on the intertidal ecosystem with a suite of research scientists, cultural practitioners, and resource managers; (2) establish a baseline survey of intertidal and shallow subtidal ecosystems, specifically focused on 'opihi species associations, relative abundance, and reproductive characteristics to better understand the implications and consequences of human impacts and natural processes on these communities; (3) characterize the biodiversity of the Hawaiian intertidal zone and their connectivity to one another across the archipelago; (4) to determine ‘opihi size at reproductive maturity and reproductive characteristics such as gonad index, and molecular composition of sperm-egg recognition proteins in the absence of human predation, (5) begin to explore the genomic signatures of adaptation to human activities and natural processes using ‘opihi as a model system to elucidate the mechanisms by which divergent selection can lead to adaptive radiation of marine species.

Objectives 1& 2: To accomplish these goals, we conduct 15-30 belt transects per island located randomly at sites selected based on access, safety and weather conditions. We also map ‘opihi density and species presence/absence at 10s to 100s of georeferenced locations on each island. The transect methodology and data collection sheets come from a series of joint retreats between the resource management agency, NGO, research scientist and cultural practitioner partners to develop the collaborative protocol we implement. The data sheet and collaborative protocol, are described in more detail in Kehau Springer's permit application. In brief, we survey a series of belt transects per island, in which teams mark the start of the transect by recording the GPS waypoints. We then lay a transect sash chain perpendicular to the shoreline (mauka to makai), from the highest marine animal on the shore to 15 ft deep. We attach colored cable ties to the sash chain to divide the transect into zones, and count all ‘opihi by size class within each zone. We count all other visually identifiable intertidal organisms associated with the ‘opihi and record the species present and the abundance of each along the transect lines. Next we estimate the percent cover of each algae species and collect a voucher specimen from each type of algae to confirm algal id later in the laboratory under a microscope. If algal turfs are present, we collect a 1cm2 voucher sample of each visually distinct turf type because turfs are typically composed of 10's of species and are not identifiable in the field. We then measure the x,y,z spatial coordinates of each zone boundary along the transect before measuring the “rugose” length of the transect laid to contour the exact surface distance of each zone. Each data sheet is double-checked and photographed in the field, and matched with a photograph of the entire transect and the conditions are recorded along with anything else noteworthy along the transect line. An additional photograph is taken every 25cm along the transect chain o capture each zone

boundary. For the mapping, six individuals survey while 2 individuals watch waves, and 2 individuals collect data. All ‘opihi are identified by species and counted, presence/absence data for invertebrates and edible limu are recorded, and a GPS coordinate is recorded and associated with the survey number in 2 m wide transects of shoreline (mauka to makai). Consecutive two meter transects are surveyed, unit by unit, until the entire accessible portion of the island is surveyed. At Mokupapapa, all opihi are counted. At Mokumanamana and Nihoa, if all sides of the island are mappable, then we survey all accessible shoreline at a boat access before moving to a new area. In 2013, we mapped all areas that the waves permitted us to survey.

We have begun conducting the first very near shore fish surveys conducted in PMNM. In order to survey shallow very near shore fish populations, we employ a system involving 4 snorkelers that swim parallel to the shore within 0-20 m of the shore line that was surveyed with transects. Each snorkeler has a different task. Snorkeler 1 surveys benthic fish. Snorkeler 2 surveys silver fish. Snorkeler 3 surveys colorful fish. Each surveying snorkeler records the number of fish of each species that they see on the swimming transect and is equipped with a camera to photograph unknown species. In 2011-12 we developed lists of the most common fish observed in the very near shore habitat that we have used to develop data sheets for data recording. Snorkeler 4 video tapes the fish along the transect to serve as visual documentation of the different species present. The snorkelers are paired and swim side by side with a boat escort for safety. The length of the swimming transect is recorded from the boat using a gps and a stopwatch to give an idea of catch per unit effort, but our primary goal at this point is to record the species present. This approach covers both objective (1) and (2), and subsequent laboratory examination of the samples will fulfill objective (3).

In order to track 'opihi reproductive cycles, we will continue to determine the gonad index of all ‘opihi collected in collaboration with Kehau Springer. The 2014 cruise will begin shortly after the full moon and end shortly after the new moon, when opihi spawn. We plan to collect 24 individuals per 1 cm size class (<1, 1-2 ,2-3 ,3-4 , 4-5, >5cm) per species on Nihoa and Mokumanamana, and transport them to the boat for immediate processing. On Mokupapapa, after confirming the census population size to be 2900 (see methods two paragraphs above), as we determined in 2013, only 5 individuals per size class will be collected (25 total) thereby ensuring that we collect fewer than 1% of the limpets present. On Puhahonu, if visited, we will use the mapping protocol to census the ‘opihi population, which is inhabited solely by *Cellana exarata*, according to previous DNA analysis. The result of the census will determine whether our ‘opihi sampling is conducted as outlined for Mokumanamana and Nihoa (>>12,000 ‘opihi on island, <<1% of population sampled), or Mokupapapa (<3000 ‘opihi on island, <1% of population sampled). RNAlater, a RNA and DNA tissue preservative, will be stored in plastic unbreakable tubes inside of plastic containment containers (5gal buckets). On the ship, we will dissect the 'opihi to separate the gonad, the non-gonadal tissue, and the shell for subsequent weighing at HIMB on O'ahu. The wet weight of the gonad and somatic tissue will be weighed and compared to assess reproductive state for males and females allowing us to estimate reproductive state. Tissues will be preserved by freezing in liquid nitrogen and storage in RNA later reagent (a solution designed for and proven to be the best method of preventing the degradation of DNA and RNA in marine invertebrate samples).

The combination of size class sampling and genomic DNA sequencing will allow us to conduct a survey of connectivity and self recruitment on an unprecedentedly fine scale in PMNM. We are still processing genetic samples from 2012 and 2013 that were collected and

processed in this vein, and we are finding genetic patterns on the scale of centimeters. One technician, four graduate students and six undergraduate students are working on processing the samples and survey data in Chris Bird's laboratory at Texas A&M.

Non-lethal sampling of crabs (*Grapsus tenuicrustatus*) and octopi (*Octopus oliveri* and *O. cyanea*) will be conducted by removing a leg, or an 1 inch section of arm tip, respectively.

In 2012, we detected a difference in the onset of reproductive maturity on Mokupapapa. Often when fishery species are managed with a size limit, the size at reproductive maturity decreases due to selective pressure for younger maturation. We are going to test this hypothesis by comparing the size at reproductive maturity for 'opihi inside and outside of PMNM. To do this, the ʻopihi listed above will be preserved and analyzed for gonad index in the laboratory with the other 'opihi.

When possible, we will extract the DNA from live invertebrate tissues on the ship at the conclusion of each day in order to avoid sample degradation that interferes with genome-wide genetic surveys. All waste will be retained on the ship, and kept in primary and secondary containment vessels. Waste will be disposed of at the University of Hawaiʻi on Oʻahu. DNA extraction involves chaotropic salts, guanidine, SDS, sodium azides, and ethanol.

Objective 3, connectivity:
As stated above, the previously employed technique of preserving tissue samples in ethanol for DNA analysis has resulted in samples that cannot be genomically analyzed. In 2012, we employed a new sample preservation method with opihi, preservation in liquid nitrogen, that was much more successful. Since then, we have found that either preservation in RNAlater reagent or extracting and stabilizing DNA from live tissue is the best method to preserve the high quality DNA required for genomic analysis, and we successfully used a field DNA extraction kit to do so. With this proven stategy, we plan to resample the other animal species that we originally collected, in 2011 and 2012 (along with the addition of *Octopus oliveri, Octopus cyanea, Actinopyga mauritiana, Heterocentrotus mammilatus, Echinothrix diadema, and Tripneustes gratilla*) for the first multispecies population genomic study in the Hawaiian Islands, and most likely the world. PIs Chris Bird and Rob Toonen have optimized genomic survey protocols. Kelly Pennoyer, Luz Angela Lopez de Mesa, and Lauren Gurski, PhD students in Chris Bird's lab, are working on this project in the main Hawaiian Islands for their dissertation and would also process the PMNM samples. 48 specimens per species per island would be collected in 2014.

The target species we have identified for genetic assessment are ones which are abundant and common on every island surveyed to date, and for which the estimated population sizes are so large that collection of 48 individuals per island will have no detectable impact. Our cut off is that we will not sample more than 1% of the population at any island, and preliminary abundance surveys from previous years indicate that populations are well in excess of 4800 individuals per island for each of the species that we have included on this permit application (with the exception of Cellana exarata on Mokupapapa, of which we will only collect 25 individuals). We will examine connectivity of the intertidal species to compare with the subtidal organisms scored to date and determined whether coral reef species are a good predictor of intertidal species connectivity. DNA samples will be analyzed using standard techniques well–established in the field and in use daily in our lab (see attached CV).

Objectives 4&5, ‘opihi reproductive characteristics and human impacts and adaptation of ‘opihi

The sampling scheme outlined above will allow us to calculate the gonad index for different size classes of opihi by determining the mass of gonads and somatic tissues. This data will allow us to determine the size at reproductive maturity and the reproductive state of opihi on different islands.

It is not very surprising that we are starting to see a strong relationship between human population size and ‘opihi population size, given the ‘opihi are actively harvested by humans. We will continue to develop a model that best describes the impact of human harvesting on ‘opihi and we will relate these results with the genetic data that we’ve been processing.

In our surveys of genomic variation, we can test for genetic loci that are related to human population and land use statistics. Patricia Cockett, a master’s student in Chris Bird’s lab is explicitly sampling opihi with respect to sources of pollution and the PMNM ‘opihi samples stand as a fantastic control group, where there is little pollution from humans.

The expressed gamete recognition protein genes will be discovered through whole transcriptome RNA sequencing of gonad tissue will be conducted on three males and three females per opihi species from Mokupapapa, Mokumanamana, and Nihoa (30 opihi sampled from the above detailed collections). DNA oligonucleotide primers will be developed to amplify these genes from the ‘opihi DNA collections developed over the past 10 years from the entire archipelago. We will test for a correlation between allele frequency and ‘opihi population density from mapping and transect surveys conducted in both PMNM and the MHI in order to identify alleles that likely confer an advantage at high or low population densities. We will also test for population structure in gamete recognition proteins among samples. A test for human impacts will be conducted by correlating gamete-recognition genes with the ratio human population size to linear coastline distance of ‘opihi habitat.

NOTE: If land or marine archeological activities are involved, contact the Monument Permit Coordinator at the address on the general application form before proceeding, as a customized application will be needed. For more information, contact the Monument office on the first page of this application

9a. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):

Common name:

Yellowfoot ‘opihi (‘opihi ‘alinalina)
Blackfoot ‘opihi (‘opihi makaiauli)
Shingle urchin (hā‘uke‘uke kaupali)
Black nerite (pipipi)
Spotted periwinkle (pipipi kōlea)
Spotted drupe (makaloa)
Black purse shell (nahawele)

Thin shell rock crab (‘a‘ama)
Rock-boring urchin (‘ina kea)
Oblong urchin (‘ina uli)
Turf algae (limu)
Octopus
Crown-of-thorns seastar
(see Appendix 1 for detailed list of samples.
Scientific name:
Cellana exarata
Cellana sandwicensis
Isognomon californicum
Smaragdinella calyculata
Grapsus tenuicrustatus
Drupa ricina
Echinolittorina hawaiiensis
Littoraria pintado
Nerita picea
Siphonaria normalis
Colobocentrotus atratus
Echinometra oblonga
Echinometra mathaei
Octopus oliveri
Octopus cyanea
Acanthaster planci

& size of specimens:

Following above, see Appendix 1 for detailed list of maximum sample sizes

Collection location:

Following above, see Appendix 1 for detailed list of collection sites

☒ Whole Organism ☒ Partial Organism

9b. What will be done with the specimens after the project has ended?

Preserved samples remain the property of the Monument, and will maintained with population preserved connectivity biopsy tissue samples collected to date at HIMB, Texas A&M, and Scripps until they are used up by the study or such time as the Monument co-trustees request that they be returned to them. Taxonomic voucher specimens will be submitted for permanent inclusion in museum collections as per the voucher specimen guidelines. Specimens will be centrally housed at Texas A&M where Chris Bird can ensure their safe storage, preservation and

care. Algae samples will be shipped to Scripps for identification and then will be housed at Texas A&M. Specimens may also be shipped to HIMB for processing. DNA and RNA samples will be processed at HIMB, Texas A&M, ARQ Genomics (Austin, TX), and Simon Frasier University. Voucher specimens will be subsampled for genetic analysis and stored frozen or in preservative prior to study.

9c. Will the organisms be kept alive after collection? ☒ No

• General site/location for collections:
0-20m at Puhahonu, Mokupapapa, Mokumanamana, and Nihoa

• Will organisms be released?
No.

10. If applicable, how will the collected samples or specimens be transported out of the Monument?

Preserved samples (frozen in RNAlater saturated salt buffer) will be transported back to Oahu aboard the vessel. Specimens will be centrally housed at Texas A&M where Chris Bird can ensure their safe storage, preservation and care. Algae samples will be shipped to Scripps for identification and then will be housed at Texas A&M. Specimens may also be shipped to HIMB for processing. DNA and RNA samples will be processed at HIMB, Texas A&M, ARQ Genomics (Austin, TX), and Simon Frasier University. Voucher specimens will be subsampled for genetic analysis and stored frozen or in preservative prior to study.

11. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research:

All researchers working on this project have coordinated to share samples and avoid duplicate sampling. Specifically, the samples listed here and those in the accompanying permit application by Kehau Springer are explicitly the same samples and not duplicative or in addition to one another.

12a. List all specialized gear and materials to be used in this activity:

We will collect samples by hand using no specialized gear or materials beyond snorkeling gear, transect lines, data sheets, and butter knives.

12b. List all Hazardous Materials you propose to take to and use within the Monument:

Tissue preservative solution for DNA and RNA analyses is RNALater, MSDS attached.. DNA extraction involves chaotropic salts, guanidine, SDS, sodium azides, and ethanol. RNAlater will

be double contained (plastic bottles inside of sealed buckets), we be used within Rubbermaid containment basins, and all waste will be double contained and disposed of at the University of Hawaii.

13. Describe any fixed installations and instrumentation proposed to be set in the Monument:

None

14. Provide a time line for sample analysis, data analysis, write-up and publication of information:

Surveys will be completed in the field during the expedition. Data analysis and write-up will depends on the availability of specific support for researchers post-cruise. With current levels of partial support and volunteer activities, we expect it will take roughly 1.5 years to complete the post-cruise analysis of survey data. Time to publication can be considerably longer since the turn-around time for some journals now exceeds 800 days, but results will be reported as soon as possible among the partners and to the resource management community.

Regardless of the time to publication, the results from these studies are made available to Monument managers as quickly as possible through the brown-bag luncheons, semi-annual reports, and semi-annual mini symposium during which all researchers involved in this project present the most current findings from their ongoing research to the broader management community. Findings are always provided to the Monument co-trustees almost as quickly as they become available, and made available to the greater management community within no more than 6 months of the data being collected. Finally, given the specific partnership of Hawaiian cultural practitioners, NGO community, State and Federal resource managers, and research scientists, we are confident that research results will be communicated widely.

Additionally this permit application is a partner to the Native Hawaiian Practices permit application of Kehau Springer. All samples and methodologies discussed in this permit application are directly related to both permit applications and are the same samples, not in addition to one another. This project and its group of dedicated participants will continue to bridge the gap between cultural and western research in Papahānaumokuākea Marine National Monument, and community participants will communicate our collective findings to their respective communities (Hana, Kalapana, etc.) as outlined in Kahau Springer's permit application.

Tentative Cruise Itinerary

DATE	PORT / Island	Departure Time	Distance	Activities
06/19/2014	Depart Kewalo for French Frigate Shoals (MPP)	0900		

06/20/2014	Transit			
06/21/2014	Transit			
06/22/2014	Arrive MPP	Full day operations at MPP		Map, Collect
06/23/2014	MPP	Full day operations at MPP		Transect, Collect
06/24/2014	MPP/Transit	½ day operations MPP, depart for MMM	155nm @ 8 knots = 20 hrs	Fish
06/25/2014	Transit/MMM	Arrive MMM, ½ day operations @ MMM		Map
6/26/2014	MMM	Full day operations @ MMM		Transect, Collect
06/27/2014	MMM/ Transit	boat Full day operations @ MMM depart for NIH around 1600	122nm @ 8 knots = 16 hrs	Map, Fish
06/28/2014	NIH	SEARCHER anchor in pm – scout coastline via small boat		Collect
06/29/2014	NIH	Full day operations @ NIH		Map, Collect
06/30/2014	NIH-Transit	Full day operations @ NIH, depart at 1600 to HNL	245nm @ 8 knots = 31 hrs	Transect, Fish
07/01/2014	Transit	Transit – arrive 11pm-ish		
07/02/2014	Transit-offload			

15. List all Applicants' publications directly related to the proposed project:

21 Simion, P, CE Bird, and RJ Toonen (in prep) Comparative phylogeography of *Octopus cyanea* and *O. oliveri* in the Hawaiian Archipelago.

20 Bird, CE, BH Holland, P Samallow, BW Bowen, and RJ Toonen (in prep) Shell game: spatially variable morphological convergence revealed by DNA analysis in sibling limpets.

19 Bird, CE, M Iacchei, and RJ Toonen (in prep) Isolation, disruptive selection, and divergence within a population of broadcast-spawning limpets.

18 Bird, CE and RJ Toonen (in prep) Patterns of recent divergence and gene flow between budding lineages and species boundaries in the sibling Hawaiian limpets (*Cellana* spp.)

17 Bird, CE, MA Timmers, PE Smouse and RJ Toonen (in review) Inferring dispersal patterns with F_{ST} and Φ_{ST} when is genetic distance is too much information? Integrative and Comparative Biology. *Invited*

16 Bird, CE, E Franklin, RJ Toonen, & CM Smith (in review) Between wave and tide marks: a unified model of water level and vertical zonation on littoral shores.

15 Bird, CE, M Stat, RD Gates, & RJ Toonen (in review) Complex analysis of molecular variance with PERMANOVA+.

14 Bird, CE, D Skillings, I Fernandez, and RJ Toonen (in press) Sympatric speciation in the post Modern Synthesis era of evolutionary biology. Evolutionary Biology. *Invited* Special issue on speciation.

13 Padilla-Gamino, JL, X Pochon, CE Bird, G Concepcion, RD Gates (in press) From parent to gamete: vertical transmission of *Symbiodinium* (Dinophyceae) in the scleractinian coral Montipora capitata.

12 Forsman, Z, B Kimokeo, CE Bird, CL Hunter & RJ Toonen (2012) Coral farming: species-specific effects of light, water motion and artificial foods. Journal of the Marine Biological Association of the UK.

11 Timmers, MA, CE Bird, DJ Skillings, PE Smouse, and RJ Toonen (2012) There's no place like home: crown-of-thorns outbreaks in the central Pacific are locally derived and independent events. PLoS ONE.

10 Bird, CE, PE Smouse, SA Karl & RJ Toonen (2011) Detecting and measuring genetic differentiation. In: S. Koenemann, C. Schubart & C. Held (eds.) Crustacean Issues: Phylogeography and Population Genetics in Crustacea. 31-73.*Invited*

9 Bird, CE (2011) Morphological and behavioral evidence for adaptive diversification of sympatric Hawaiian limpets. Journal of Integrative and Comparative Biology. 51:466-473.

8 Bird, CE, B Holland, BW Bowen & RJ Toonen (2011) Diversification of sympatric broadcast-spawning limpets (*Cellana* spp.) within the Hawaiian archipelago. Molecular Ecology. 20:2128-2141.

7 Gaither, MR , Z Szabo, M Crepeau, CE Bird & RJ Toonen (2011) Preservation of corals in salt-saturated DMSO buffer is superior to ethanol for PCR experiments. Coral Reefs. 30: 329-333.

6 Toonen, RJ, K Andrews, I Baums, CE Bird, G Concepcion, T Daly-Engel, J Eble, A Faucci, M Gaither, M Iacchei, J Puritz, J Schultz, D Skillings, M Timmers & B Bowen (2011) Defining boundaries for ecosystem-based management: a multispecies case study of marine connectivity across the Hawaiian archipelago. Journal of Marine Biology Article ID 460173, 15pp. doi:10.1155/2011/460173

5 Stat, M, CE Bird, X Pochon, L Chasqui, LJ Chauka, GT Concepcion, D Logan, M Takabayashi, RJ Toonen & RD Gates (2011) Variation in *Symbiodinium* ITS2 sequence assemblages among coral colonies. PLoS ONE 6(1): e15854. doi:10.1371/journal.pone.0015854

4 Skillings, D, CE Bird & RJ Toonen (2011) Gateways to Hawai'i – genetic population structure of the tropical sea cucumber *Holothuria atra*. Journal of Marine Biology. Article ID 783030, 16 pp

3 Timmers, MA, K Andrews, CE Bird, MJ deMaintenon, RE Brainard & RJ Toonen (2011) Widespread dispersal of the crown-of-thorns sea star, *Acanthaster planci*, across the Hawaiian Archipelago and Johnston Atoll. Journal of Marine Biology. Article ID 934269, 10 pp. doi:10.1155/2011/934269

2 Rodgers, KS, PL Jokiel, CE Bird, & EK Brown (2009) Quantifying the condition of Hawaiian coral reefs. Aquatic Conservation. 20:93-105.

1 Bird, CE, BS Holland, BW Bowen & RJ Toonen (2007) Contrasting phylogeography in three endemic Hawaiian limpets (*Cellana* spp.) with similar life histories. Molecular Ecology. 16:3173-3187.

With knowledge of the penalties for false or incomplete statements, as provided by 18 U.S.C. 1001, and for perjury, as provided by 18 U.S.C. 1621, I hereby certify to the best of my abilities under penalty of perjury of that the information I have provided on this application form is true and correct. I agree that the Co-Trustees may post this application in its entirety on the Internet. I understand that the Co-Trustees will consider deleting all information that I have identified as "confidential" prior to posting the application.

_____ _____

Signature Date

SEND ONE SIGNED APPLICATION VIA MAIL TO THE MONUMENT OFFICE BELOW:

Papahānaumokuākea Marine National Monument Permit Coordinator
6600 Kalaniana'ole Hwy. # 300
Honolulu, HI 96825
FAX: (808) 397-2662

DID YOU INCLUDE THESE?

☒ Applicant CV/Resume/Biography
☒ Intended field Principal Investigator CV/Resume/Biography
☒ Electronic and Hard Copy of Application with Signature
☒ Statement of information you wish to be kept confidential
☒ Material Safety Data Sheets for Hazardous Materials

Appendix 1. Maximum total collection numbers.

June 2014 Collection List

Species	Type	Distribution	Sampling	Nihoa	Mokumanamana	Mokupāpapa (French Frigate Shoals)	Pūhāhonu (Gardner Pinnacles0	Preserve Tissue For Genetic Analysis
Isognomon californicum	Bivalve	Hawaii	Lethal	48	48	48	48	Y
Smaragdinella calyculata	Bubble Shell	Indo-Pac	Lethal	48	48	48	48	Y
Grapsus tenuicrustatus	Crab	Indo-Pac	Non-lethal	48	48	48	48	Y
Octopus oliveri	Octopus	Indo-Pac	Non-Lethal	48	48	48	48	Y
Octopus cyanea	Octopus	Indo Pac	Non-Lethal	48	48	48	48	Y
Drupa ricina	Snail	Indo-Pac	Lethal	48	48	48	48	Y
Echinolittorina hawaiiensis	Snail	Hawaii	Lethal	48	48	48	48	Y
Littoraria pintado	Snail	Indo-Pac	Lethal	48	48	48	48	Y
Nerita picea	Snail	Hawaii	Lethal	48	48	48	48	Y
Siphonaria normalis	Limpet	Indo-Pac	Lethal	48	48	48	48	Y
Cellana exarata	Limpet	Hawaii	Lethal	144	144	< 1% of pop, up to 25	<1% of pop, up to 144	Y
Cellana sandwicensis	Limpet	Hawaii	Lethal	144	144	0	0	Y
Colobocentrotus atratus	Urchin	Indo-Pac	Lethal	48	48	48	48	Y
Echinometra oblonga	Urchin	Indo-Pac	Lethal	48	48	48	48	Y
Echinometra mathaei	Urchin	Indo-Pac	Lethal	48	48	48	48	Y
Acanthaster planci	Sea star	Indo-Pac	Lethal	48	48	48	48	Y
Misc Algae	algae	various	Non-lethal	1000	1000	1000	1000	Y