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Status Review Report:
Orange Clownfish (*Amphiprion percula*)



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National Marine Fisheries Service
National Oceanic and Atmospheric Administration
U.S. Department of Commerce

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ABSTRACT

This report was produced in response to a petition received from the Center for Biological Diversity on September 14, 2012, to list eight species of pomacentrid reef fish as endangered or threatened under the Endangered Species Act (ESA) and to designate critical habitat for these species concurrent with the listing. The National Marine Fisheries Service (NMFS) evaluated the petition to determine whether the petitioner provided substantial information as required by the ESA to determine that listing these species may be warranted. On September 3, 2014, the NMFS Pacific Islands Regional Office (PIRO) announced in the Federal Register that the petition presented substantial information that listing may be warranted for the orange clownfish (*Amphiprion percula*), and NMFS requested information on this species from the public (79 FR 52276). Subsequently, NMFS initiated a status review of this species, which we document in this report. This report summarizes the best available scientific and commercial information on the orange clownfish, and presents an evaluation of the species' status and extinction risk.

On September 3, 2014, NMFS PIRO also announced a negative 90-day finding for the six Indo-Pacific damselfishes: Hawaiian dascyllus (*Dascyllus albisella*), blue-eyed damselfish (*Plectroglyphidodon johnstonianus*), black-axil chromis (*Chromis atripectoralis*), blue-green damselfish (*Chromis viridis*), reticulated damselfish (*Dascyllus reticulatus*), and blackbar devil or Dick's damselfish (*Plectroglyphidodon dickii*). The NMFS Southeast Regional Office led the response to the petition to list the yellowtail damselfish (*Microspathodon chrysurus*) and announced a negative 90-day finding (80 FR 8619) for that species on February 18, 2015.

In assessing four demographic risks for *A. percula* -- abundance, growth rate/productivity, spatial structure, and diversity -- we determined that the likelihood of these risks individually contributing to the extinction risk for the species is low or unknown. We also assessed current and predicted threats to the species and determined that the likelihood of these individual threats contributing to the extinction risk of the species throughout its range varies between very low and low-to-medium. We acknowledge that uncertainties exist regarding how these demographic risks and current and predicted threats may affect the species at both the individual and population levels.

Of the 12 identified current and predicted threats, our greatest concern relates to the species' susceptibility and exposure to sedimentation and nutrients, as well as the inadequacy of regulatory mechanisms to address this threat, especially since juveniles and adults occur in shallow water and are non-migratory once they have settled into a host anemone. Therefore, we conservatively assigned a low-to-medium likelihood that both this threat and the inadequate regulatory mechanisms to address this threat may significantly contribute to the extinction risk for *A. percula*.

The range of the species across heterogeneous habitats, the conservatively estimated abundance of 13-18 million individuals, the spatial and temporal variation in threats, coupled with resiliency and potential for trans-generational adaptive capabilities to future impacts all contribute to a low overall vulnerability of the species to the collective threats we have identified. We have determined that the overall extinction risk to *A. percula* is low, both now and in the foreseeable future.

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INTRODUCTION AND BACKGROUND

Scope and Intent of this Document

This report documents the status review conducted in response to a petition¹ to list the orange clownfish (*Amphiprion percula*) under the Endangered Species Act (ESA). Under the ESA, if a petition is found to present substantial scientific or commercial information that the petitioned action may be warranted, a status review shall be promptly commenced (16 U.S.C. 1533(b)(3)(A)). The National Marine Fisheries Service (NMFS or NOAA Fisheries) determined that the petition presented substantial information that a status review was warranted for the orange clownfish (79 FR 52276; September 3, 2014), and promptly initiated a status review. The ESA stipulates that listing determinations should be made based on the best scientific and commercial information available, after taking into consideration any efforts by any State or foreign nation, or any political subdivision of a State or foreign nation, to protect the species (16 U.S.C. §1533(b)).

NMFS assigned two Endangered Species Biologists in the Protected Resources Division of the NMFS Pacific Islands Regional Office (PIRO) to compile the best available data on this species, and complete a thorough review of the biology, population status, and future outlook for this species. An extensive literature search was undertaken and researchers were contacted regarding gray literature and additional information. As announced in the 90-day finding, NMFS also solicited the public for relevant data and information from September 3, 2014, through November 3, 2014. Relevant information submitted by the public, contributed by experts, and extracted from the literature search is incorporated into this status review.

This status review includes an analysis of the biology, demography, and ecology of the species, threats to the species, and makes conclusions regarding the extinction risk of the species. For the risk assessment, we used a qualitative reference level of relative extinction risk modified from the reference levels commonly used in status reviews (e.g., **rockfish in the Puget Sound, Banggai cardinal fish**, etc.). Recommendations as to whether the species should be listed as threatened or endangered were not made. Rather, conclusions are drawn about the overall risk of extinction faced by the species based on an evaluation of the species' current status, demographic risks, as well as present and future threats to the species and how the species is responding, or is likely to respond in the future, to those threats.

Questions and Information Considered in ESA Evaluations

In determining whether a listing under the ESA is warranted, two key questions must be addressed:

- 1) Is the entity in question a “species” as defined by the ESA?
- 2) If so, is the “species” threatened or endangered?

¹ Center for Biological Diversity to U.S. Secretary of Commerce, Acting through the National Oceanic and Atmospheric Administration and the National Marine Fisheries Service, September 14, 2012, “Petition to list 8 species of Pomacentrid reef fish under the U.S. Endangered Species Act.”

Section 3 of the ESA defines a “species” to include “any subspecies of fish or wildlife or plants, and any distinct population segment (DPS) of any species of vertebrate fish or wildlife which interbreeds when mature.” Section 3 further defines the term “endangered species” as “any species which is in danger of extinction throughout all or a significant portion of its range.” The term “threatened species” is defined as “any species which is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range.”

NMFS considers a variety of information in evaluating the level of risk faced by a species in deciding whether the species meets the statutory definition of either threatened or endangered. Important considerations include 1) absolute numbers of individuals and their spatial and temporal distribution, 2) current abundance in relation to historical abundance and carrying capacity of the habitat, 3) trends in abundance, 4) natural and human influenced factors that cause variability in survival and abundance, 5) possible threats to genetic integrity, and 6) recent events (e.g., a change in management) that have predictable short-term consequences for abundance of the species. Additional risk factors, such as disease prevalence or life history traits, may also be considered in evaluating risk to populations (NMFS 2013).

Under section 4(a)(1) of the ESA, NMFS must determine whether one or more of the following factors is/are causing a species to be threatened or endangered:

- (A) The present or threatened destruction, modification or curtailment of its habitat or range;
- (B) Overutilization for commercial, recreational, scientific, or educational purposes;
- (C) Disease or predation;
- (D) The inadequacy of existing regulatory mechanisms; or
- (E) Other natural or human factors affecting its continued existence.

The determination of whether a species is threatened or endangered must be based on the best available scientific and commercial information regarding its current status, after taking into consideration measures in place to conserve the species. The purpose of this document is to review and summarize the best available information/data to describe the status of the orange clownfish. A determination as to whether or not the species meets the statutory definition of threatened or endangered, and therefore may be warranted for listing, is not included in this document but will be included in the 12-month finding for the species.

LIFE HISTORY AND ECOLOGY

Classification and Distinctive Characteristics

The orange clownfish, *Amphiprion percula*, is a valid taxonomic species within the family Pomacentridae. The species was first described by Lacepède in 1802, as *Lutjanus percula* and later re-described as *Amphiprion percula* (Florida Museum of Natural History 2005). There are 360 species in the Pomacentridae family that are classified into 29 genera. Two of those genera contain all 28 recognized species of clownfish: one species in the genus *Premnas*, and the remaining species in the genus *Amphiprion*. The number of recognized clownfish species has

evolved over time due to inconsistent recognition of natural hybrids and geographic color variants of previously described species as separate species in the literature (Allen 1991; Fautin and Allen 1997; Buston and Garcia 2007; Ollerton *et al.* 2007; Allen *et al.* 2008; Thornhill 2012; Litsios *et al.* 2014; and Tao *et al.* 2014). Their mutualistic relationship with sea anemones is correlated with the adaptive radiation and accelerated speciation of clownfish species (Litsios *et al.* 2012). In addition, hybridization events are linked with diversification in clownfish, and several recently diverged clownfish lineages likely originated through hybridization. This suggests that diversification, catalyzed by hybridization events, may still be happening (Litsios and Salamin 2014).

The taxonomic classification for *A. percula* is as follows:

Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii
Order:	Perciformes
Family:	Pomacentridae
Genus:	<i>Amphiprion</i>
Species:	<i>percula</i>

The species is known by common English names that include orange clownfish, clown anemonefish, percula clownfish, percula anemonefish, orange anemonefish, true percula clownfish, blackfinned clownfish, eastern clownfish, eastern clown anemonefish, and orange-clown anemonefish (Animal-World 2015). Common names in other languages include bantay bot-bot (Cebuano); orangegul klovnfisk (Danish); pata (Davawenyo); maumanu ni masao (Gela); clownfisch (German); samok-samok (Kagayanen); paja-paja (Makassarese); clown fish biak, gelang roay (Malay); amfiprion (Polish); baro-baro (Visayan); and bantay-kibot (Waray-waray) (Florida Museum of Natural History 2005). A more comprehensive list of common names can be found at the Fishbase web site (www.Fishbase.org).

Amphiprion percula is bright orange with three thick white vertical bars (Fig. 1). The anterior bar occurs just behind the eye, the middle bar bisects the fish and has a forward-projecting bulge, and the posterior bar occurs near the caudal fin. The white bars have a black border that varies in width. Orange clownfish have 30-38 pored scales with no interruptions along the lateral line, and their fins have black tips (Fautin and Allen 1997; Florida Museum of Natural History 2005). Although this describes the type specimen, some polymorphism does occur with diverse geographic regional and local color forms, mostly in the form of variation in the width of the black margin along the white bars (Militz 2015; Timm *et al.* 2008).

While there is no difference in color pattern between sexes, dimorphic variation is present in size as females are larger than males (Fautin and Allen 1997; Florida Museum of Natural History 2005). It is important to note that size alone cannot be used to identify the sex of an individual because individuals in different groups will vary in maximum and minimum size. Maximum reported length for this species is approximately 80 millimeters (mm) (Fautin and Allen 1997), but individuals up to 110 mm in length have been reported (Florida Museum of Natural History 2005). Standard length is reported as 46 mm for females and 36 mm for males (Florida Museum of Natural History 2005). The total length of a fish has been correlated with the diameter of its host anemone (Fautin 1992), with larger anemones hosting larger clownfish.

Amphiprion percula very closely resembles *A. ocellaris*, also known as the false percula clownfish, and the two are considered sibling species. There are several morphological differences that may allow an observer, upon closer examination, to distinguish between the two species. *Amphiprion percula* has 9-10 dorsal spines while *A. ocellaris* has 10-11 (Timm *et al.* 2008), and the anterior part of *A. percula*'s dorsal fin is shorter than that of *A. ocellaris*. In addition, *A. percula* has a thick black margin around its white bars whereas *A. ocellaris* often has a thin or even non-existent black margin, though this is not always the case. *Amphiprion percula* has been described as more brilliant in color, and its iris is orange, giving the appearance of very small eyes while the iris of *A. ocellaris* is grayish-orange, thus giving the appearance of slightly larger eyes (Florida Museum of Natural History 2005). Ecologically, both species prefer some of the same host anemone species (*Heteractis magnifica*; *Stichodactyla gigantea*; *S. mertensii*) (Fautin and Allen 1997; Timm *et al.* 2008).

Of noted difference is that these two species have an allopatric distribution, meaning their ranges do not overlap. *Amphiprion percula* is found in northern Queensland and Melanesia; *A. ocellaris* is found in the Andaman and Nicobar Islands (Andaman Sea), Indo-Malayan Archipelago, Philippines, northwestern Australia, and the coast of Southeast Asia northwards to the Ryukyu Islands of Japan (Fautin and Allen 1997; Timm *et al.* 2008). Genetically, the two species appear to have diverged between 1.9 and 5 million years ago (Litsios *et al.* 2012; Nelson *et al.* 2000; Timm *et al.* 2008). In the aquarium trade, *A. ocellaris* is the most popular anemonefish with *A. percula* the second most popular (Animal-World 2015). The two species are often mistaken for one another and misidentified in the aquarium trade. They are also often reported as a species complex (i.e., reported as *A. ocellaris/percula*) in trade documentation and scientific research due to the difficulty in distinguishing between the two species. Even though their ranges do not overlap, source countries often catch and/or culture one or both species, exporting both wild-captured and captive bred individuals.

Habitat

Amphiprion percula is described as a habitat specialist due to its symbiotic association primarily with three species of anemone: *Heteractis crispa*, *H. magnifica*, and *Stichodactyla gigantea* (Fautin and Allen 1997; Elliott and Mariscal 1997a; Ollerton *et al.* 2007). The species has also been reported as associating with *S. mertensii* (Elliott and Mariscal 2001) and *S. haddoni* (Planes *et al.* 2009). As described in more detail below in the Geographic Range section, anemone habitat for *A. percula* is spread throughout northern Queensland (Australia), the northern coast of West Papua, northern Papua New Guinea (including New Britain), the Solomon Islands, and Vanuatu (Rosenberg and Cruz 1988; Fautin and Allen 1997; De Brauwer 2014) (Fig. 2).

Anemones and their symbiotic anemonefish inhabit coral reefs and nearby habitats such as lagoons and seagrass beds. Although Fautin and Allen (1992, 1997) estimate that as many anemone hosts and symbiotic fish live on sand flats or other substrate surrounding reefs as live on the reef itself, the symbiotic pairs are thought of as reef dwellers because most diving and observations occur on reefs. Both symbionts reside in shallow coastal waters primarily in depths of 1-12 meters (m) (though the anemones can be found in depths up to 50 m) and water temperatures ranging from 25-28 °C (77-82 °F) (Fautin and Allen 1997; Randall *et al.* 1997). Two anemone species, including one *A. percula* host (*H. crispa*), and two species of symbiotic

anemonefish have been reported from mesophotic depths (>50m) in Australia (Bridge *et al.* 2012).

Although anemonefishes have been the subject of considerable scientific research, less is known about the population dynamics or biology of the giant anemones that serve as their hosts. (Anemones and giant anemones are used interchangeably here and in the literature; several individual anemones may cluster together, forming what appears as a giant individual (Fautin and Allen 1997).) There are over 1,000 anemone species but only 10 of them are known to be associated with anemonefish. As described by Fautin and Allen (1992, 1997), giant anemones have a lower end known as the pedal, which attaches firmly to a solid object like a coral branch or rock. The opposite, unattached end is the mouth, or oral disc. Hollow tentacles emerge from the oral disc and come in varying shapes, lengths, sizes, and colors. Microscopic, single-celled algae known as zooxanthellae live within the tentacles and oral disc and provide energy to the anemone through photosynthesis. Microscopic nematocysts, or stinging cells, are found on the tentacles and internal structures and are used for defense and to capture prey (Fautin and Allen 1997). Acting like **microscopic harpoons or needles, nematocysts mechanically sting prey. Nematocyst toxins cause pain, loss of muscular coordination, paralysis, and tissue damage (Mebs 1994, 2009).** Prey items include plankton, small fish, sea urchins, and crustaceans such as shrimp and crabs. **The mucous coating of giant anemones also contains cytolytic poisons, which are lethal at dilute concentrations to most fish (Mebs 1994, 2009).**

Relatively little is known about reproduction in giant anemones. Male and female *H. crispa* anemones synchronously broadcast spawn sperm and eggs into the water column a few nights each year (Scott and Harrison 2007a). After spawning, fertilized eggs become ciliated planula larvae and become motile within 36 hours (Scott and Harrison 2007b). The larvae disperse for 4-12 days (Scott and Harrison 2007b, 2008) **before settling in appropriate habitat. High mortality is likely associated with this larval stage, as is common with broadcast spawning species, although dispersal distances and mortality rates have not been examined for any giant anemone species (Thornhill 2012).** In addition to reproducing sexually, *H. magnifica* is also able to reproduce asexually, whereby a polyp divides and becomes two polyps within the space of a few days. Each of the two smaller individuals continues to grow before dividing again (Fautin and Allen 1997). It is unknown which form of reproduction (i.e., sexual vs. asexual) is more common.

Giant anemones are likely slow growing and very long lived, living decades to several centuries (Fautin 1991; Fautin and Allen 1997). To be a viable host for anemonefish, an anemone must be of a sufficient size to provide shelter and protection from predators. The long-term growth rate and survival of anemones is correlated with the size and number of anemonefish they host, which provide protection for the anemone from predators (Porat and Chadwick-Furman 2004) among the other benefits listed below. As for locomotion, anemones are typically settled at their location, though if conditions are unfavorable, they are able to use their pedal disc to move a few millimeters a day, or may detach entirely and roll or be carried a longer distance via water currents (Fautin and Allen 1997).

The symbiosis between *A. percula* and its host anemones serves as an effective anti-predation measure for both symbionts. Clownfish, including *A. percula*, are a unique group of fish that can live unharmed among the stinging tentacles of anemones. A thick mucus layer cloaks the fish

from detection and response by anemone tentacles (Rosenberg and Cruz 1988; Elliott and Mariscal 1997a, 1997b). Species that lack this physiological adaptation are immobilized by stinging tentacles and consumed by the anemone. Thanks to this symbiotic association and protection from their host anemones, adult *A. percula* have very few predators. Predators of both anemones and anemonefish are deterred by the anemone's stinging tentacles and by the presence of territorial clownfish. In return, anemonefish swim through and create fresh water circulation for the stationary anemone, allowing it to access more oxygenated water, speed up its metabolism, and grow faster (Szczebak *et al.* 2013). Anemonefish also fertilize host anemones with their ammonia-rich waste (Roopin and Chadwick 2009; Cleveland *et al.* 2011)), leading to increases in anemone growth and asexual reproduction (Holbrook and Schmitt 2005).

At most geographic locations where anemonefish populations have been studied, all or most anemones are occupied by anemonefish (Mariscal 1970; Allen 1972; Fautin 1985, 1992; Ochi 1986; Hattori 1995; Elliott and Mariscal 2001). Unoccupied anemones are typically either very small or in shallow water (Elliott and Mariscal 2001) (~<1 m), presumably due to the potential for lower salinity, increased temperatures, and exposure at low tides. Typically, only one species of anemonefish occupies a single anemone at any given time due to niche differentiation; but this is not always the case. Elliott and Mariscal (2001) studied anemone use by anemonefish in the region of Madang, Papua New Guinea, which has the highest reported species diversity of both anemonefish (9 species) and their host anemones (10 species). Of 72 anemones occupied by *A. percula*, 4 also hosted other anemonefish species (Elliott and Mariscal 2001). As a demonstration of further niche differentiation, both *A. percula* and *A. perideraion* occupied *H. magnifica* anemones, but *A. percula* occupied the *H. magnifica* individuals in nearshore zones, while *A. perideraion* occupied *H. magnifica* in offshore zones.

Amphiprion percula is a highly territorial species, likely due to intense competition for limited resources, with niche differentiation caused by the distribution, abundance, and recruitment patterns of competing species (Fautin and Allen 1997; Elliott and Mariscal 1997a, 2001; Randall *et al.* 1997). Once anemonefish settle into a host, they are unlikely to migrate between anemones (Mariscal 1970; Elliott *et al.* 1995).

Diet, Feeding, and Growth

Anemonefish are omnivorous and feed on a variety of prey items consisting of planktonic algae and zooplankton, such as copepods and larval tunicates (Fautin and Allen 1997). *Amphiprion percula* also feeds on prey remnants left over from its host anemone's feeding activity and dead tentacles from its host (Fautin and Allen 1997; Florida Museum of Natural History 2005).

An anemone will typically host a female and male breeding pair and up to four other subordinate, non-breeding and non-related percula clownfish (Buston 2003a; Buston and Garcia 2007; Buston *et al.* 2007). Individuals rarely stray beyond the periphery of their anemone's tentacles to feed (Buston 2003c). A size-based hierarchy develops within each group; the female is the largest (rank 1), the male second largest (rank 2), and the non-breeding males get progressively smaller as you descend the hierarchy (ranks 3-6) (Allen 1991). Subordinates tend to be 80% of the size of their immediate dominant in the hierarchy (Buston 2003b; Buston and Cant 2006). Subordinates likely regulate their growth to avoid coming into conflict with their immediate dominant, and thereby avoid eviction from the social group (Buston 2003b; Buston and Wong 2014). When a fish is removed from the hierarchical social group structure (due to

mortality or collection), all smaller members grow rapidly, filling in the size gap, to the point that they are once again 80% the size of their immediate dominant (Fautin and Allen 1997; Buston 2003b).

Reproduction and Development

Spawning for *A. percula* can occur year round due to perpetually warm waters within the species' range (Fautin and Allen 1997). Spawning is also strongly correlated with the lunar cycle, with most nesting occurring when the moon is full or nearly so (Fautin and Allen 1997).

Like all anemonefishes, all *A. percula* are born as males (Fautin and Allen 1997). Females develop through protandrous hermaphroditism, or sex change from male to female. This occurs when the female and largest member of the group dies (or is otherwise removed) and the next largest male changes sex to become the dominant breeding female. The second largest male subsequently becomes the dominant male (Rosenberg and Cruz 1988; Fautin and Allen 1997). Only the dominant pair contributes to the reproductive output of a group within an anemone. Non-breeders within the social group do not have an effect on the reproductive success of mating pairs (Buston 2004; Buston and Elith 2011). Within a social group, the rate of development from juvenile to adult and male to female is highly dependent on the mortality rates of individuals within the group (Fautin and Allen 1997; Buston 2004b). In other words, the larger the group size (up to six individuals), the longer an individual fish could remain as a sub-adult male (i.e., subordinate to the breeding female and male pair).

Courtship

Adult male and female *A. percula* form strong monogamous pair-bonds. Initiation of ritualized courtship is highly correlated with the lunar cycle (Fautin and Allen 1997) and typically commences three to five days prior to spawning. Social interaction increases noticeably during courtship and includes chasing and dorsal fin erection (Fautin and Allen 1997). During this time, the female's belly begins to swell with eggs (Animal-World 2015). Courtship also includes preparation of the nest site, which is important for survival of the eggs. This is done by the mating male through nipping at the host anemone's tentacles in order to create a flat patch of rock located under the anemone (Rosenberg and Cruz 1988; Myers 1999), and using his mouth to clear any algae or debris (Fautin and Allen 1997; Buston and Elith 2011).

Spawning

Once the female is ready to spawn, a conical whitish tube known as an ovipositor becomes visible and eggs slowly descend from her belly onto the nest site. Once eggs are laid, the male follows closely behind and fertilizes them externally. Spawning can last anywhere from 30 minutes to 2 hours (Fautin and Allen 1997; Dhaneesh *et al.* 2009). Clutch sizes vary widely between 100 to over 1000 eggs (Fautin and Allen 1997; Dhaneesh *et al.* 2009), with the average being 324 eggs \pm 153 (mean \pm one standard deviation) recorded in Madang Lagoon, Papua New Guinea (Buston and Elith 2011), depending on fish size and previous experience. Larger and more experienced mating pairs will produce more eggs per clutch (Fautin and Allen 1997; Buston and Elith 2011; Animal-World 2015), and can produce up to three clutches per lunar cycle (Gordon and Hecht 2002; Buston and Elith 2011). Buston (2004) reports 12 years as the typical expected tenure of breeding females in the field. While this is relatively long for a fish

species of this small size, it is not unheard of among reef fish species (e.g., 13 years for *A. clarkii* (Moyer 1986); 18 years for *A. frenatus* and 18+ years for *A. perideraion* (Fautin and Allen 1997)). Reports of clownfish in captivity conclude that clownfish cease spawning several years before the end of their life expectancy (Animal-World 2015), but it is unknown if this is also the case in the wild.

Development

After egg deposition has finished, a 6-8 day incubation period begins, with developmental rate varying with temperature and oxygen content of the water (Dhaneesh *et al.* 2009). Eggs are adhered to the nest surface via short filaments (Fautin and Allen 1997; Dhaneesh *et al.* 2009) and are attended to by the male. The male aerates the nest with his mouth and fans the eggs with his pectoral fins (Fautin and Allen 1997; Dhaneesh *et al.* 2011). The male also removes dead eggs and debris from the nest, ensuring ample oxygenation. Though the overhanging tentacles of the anemone provide some protection (Allen 1991; Arvedlund *et al.* 2000), the male fiercely guards the eggs against potential predators (Rosenberg and Cruz 1988). The most common egg predators during the day are *Pseudochromis fuscus* wrasses (family Labridae) and other damselfishes (family Pomacentridae); nighttime predators are typically not fish, but invertebrates such as brittle stars (families Ophiotrichidae, Ophiochimidae, and Ophiodermatidae) (Arvedlund *et al.* 2000 citing D.R. Bellwood, pers. comm.). During this period of male parental care, the female is occupied with feeding activity (Fautin and Allen 1997).

As eggs continue to incubate over the course of a week, embryos become visible through a transparent membrane. Silvery pupils contained within large eyes and red-orange yolk sacs are two noticeable features during development (Buston and Elith 2011). Average hatch success recorded in Madang Lagoon, Papua New Guinea, is estimated at 87% (Buston and Elith 2011). Upon hatching, which typically occurs in the evening, larvae are 3-4 mm in length (Fautin and Allen 1997; Gordon and Hecht 2002; Dhaneesh *et al.* 2009). They initially sink to the benthic environment before swimming to the upper surface of the water column using a process called phototaxis where moonlight helps to orient them (Fautin and Allen 1997). Larvae then enter a pelagic phase and are likely engaged in active swimming and orientation, and also transported by ocean currents (Fautin and Allen 1997; Leis *et al.* 2011).

Settlement and Recruitment

The larval stage of *A. percula* ends when the larval anemonefish settles into a host anemone approximately 8-12 days after hatching (Fautin and Allen 1997; Almany *et al.* 2007; Buston *et al.* 2007). Compared to many other coral reef species, this is considered a short pelagic larval duration, though is typical of genera restricted to the Indo-Pacific (Wellington and Victor 1989; Almany *et al.* 2007). The likelihood of successful recruitment depends on anemone saturation and habitat type (Buston 2003c; Dixson *et al.* 2008; Elliott and Mariscal 2001). Buston (2003c) showed that removal of non-breeders resulted in recruitment of new individuals within a few months. Under natural conditions, populations are close to carrying capacity and resident *A. percula* prevent recruitment of new individuals; when residents are removed, new individuals recruit. Like most reef fish species, the egg, pelagic larval, and settlement life stages for *A. percula* experience the highest rates of natural mortality (there may be only one successful recruit for every 10,000 eggs produced – Peter Buston pers. obs.). Once they gain the protection of an anemone, mortality is greatly reduced.

Anemonefishes search for and settle into a suitable host anemone using a variety of cues. Dixson *et al.* (2008, 2014) and Munday *et al.* (2009a) found that *A. percula* are attracted to olfactory cues such as leaf litter and tropical trees, a means of locating island reef habitats, when searching for a settlement site. *Amphiprion percula* are also attracted to unrelated conspecific adults (Munday *et al.* 2009a). Embryos and newly hatched juveniles may learn cues from the host anemone where they hatched and respond to imprinted cues when searching for suitable settlement locations (Fautin and Allen 1997; Arvedlund *et al.* 2000; Paris *et al.* 2013; Dixson *et al.* 2014; Miyagawa-Kohshima 2014). Innate recognition is also used and refers to the ability of anemonefish to locate a suitable host without prior experience (Fautin and Allen 1997; Miyagawa-Kohshima 2014). Studies indicate that imprinting on anemone olfactory cues complements innate recognition, leading to rigid species-specific host recognition (Miyagawa-Kohshima 2014).

Fish acclimation to a host anemone lasts anywhere from a few minutes to a few hours (Fautin and Allen 1997; Arvedlund *et al.* 2000) and is accomplished by briefly touching the tips of the tentacles with pectoral fins. At first there is an adhesive effect and the fish typically flees the anemone but then continually returns, making progressively more contact until tentacular clinging no longer occurs due to a protective mucus coating that develops on the anemonefish (Davenport and Norris 1958; Elliott and Mariscal 1997a). Once acclimated, the mucus protection may disappear upon extended separation between host and fish. Continued contact with tentacles appears to reactivate the mucus coat (Arvedlund *et al.* 2000). Coloration of anemonefish usually also begins during this anemone acclimation process (Elliott and Mariscal 2001). Upon settlement, the entire metamorphosis from larva to juvenile takes about a day (Fautin and Allen 1997).

Longevity and Resilience

Fautin and Allen (1992, 1997) reported that some anemonefish are recorded to have lived at least 6-10 years in nature, with 18 years the record in captivity. More recently, Buston and Garcia (2007) studied a wild population of *A. percula* in Papua New Guinea and their results suggest that females can live up to 30 years in the wild (lower 95% CI = 22.0 years, upper 95% CI = 89.9 years), although this life expectancy estimate has not been empirically proven through otolith examination. This estimate is notably two times greater than the longevity estimated for any other coral reef damselfish and six times greater than the longevity expected for a fish that size (Buston and Garcia 2007). Their results are consistent with the idea that organisms subject to low levels of extrinsic mortality, like anemonefish, experience delayed senescence and increased longevity (Buston and Garcia 2007). For individuals that move up in social rank to eventually attain the female position, remaining life span or time spent as a female does not vary with group size. The amount of time spent queuing to attain the female position, however, does vary with group size, which leads to the variable overall life expectancy of a female based on group size (Buston and Garcia 2007).

As noted above, post-recruitment mortality associated with predation is quite low, ranging from 2% (Elliott and Mariscal 2001) to ~7% for ranks 1-3 and ~30% for ranks 4-6 (Buston 2003a), especially compared to other coral reef fish. This is likely attributable to the amount of protection provided by host anemones (Buston 2003a). Social rank appears to be the only factor associated with mortality of the species post-settlement rather than factors such as number of

individuals, density, standard length, anemone diameter, depth, or reef size (Buston 2003a, 2003b). Competition for rank amongst individuals within an anemone can lead to eviction of subordinates. Buston (2003a) speculated that individuals probably compete for rank because it confers access to reproduction, and not because it confers access to food or shelter.

Using a methodology designed to determine resilience to fishing impacts, Fishbase.org rates *A. percula* as highly resilient, with an estimated minimum population doubling time of less than 15 months (Florida Museum of Natural History 2005; Fishbase.org 2015). Another analysis, using the Cheung *et al.* (2005) “fuzzy logic” method for estimating fish vulnerability to fishing pressure, assigned *A. percula* a low vulnerability score, with a level of 23 out of 100 (Fishbase.org 2015). Fujita *et al.* (2014) analyzed data deficient marine aquarium trade species, although they did not include *A. percula*. Using a recently developed semi-quantitative methodology known as the productivity susceptibility analysis (Patrick *et al.* 2010), *A. ocellaris* and *A. clarkii* were assessed and both were rated as highly vulnerable due to their low productivity and high susceptibility to capture for the aquarium fishery. While this is informative, it is difficult to determine if these results are indicative of the status of *A. percula* as well due to several important differences including the species’ range and levels of exploitation in countries where they occur. While all of these analyses are specific to the threat of fishing pressure (which is not the only threat facing *A. percula*), they assess the life history characteristics of the species (or similar species) and determine how those characteristics may affect its response to population reduction, which is informative in the case of sources of mortality besides fishing pressure. As such, we consider the species-specific results to be reliable indicators of the species’ resilience overall.

Geographic Range

Clownfish first appeared and diversified in the Indo-Australian Archipelago (Litsios *et al.* 2014). The orange clownfish is native to the Indo-Pacific region (Rosenberg and Cruz 1988), from 6° S - 26° S, 141° E - 155° E. Range countries include northern Queensland (Australia), the northern coast of West Papua, northern Papua New Guinea (including New Britain), the Solomon Islands, and Vanuatu (Rosenberg and Cruz 1988; Fautin and Allen 1997; De Brauwer 2014) (see Fig. 2). There are reports of *A. percula* occurring beyond these areas, including in the Andaman and Nicobar Islands off southeast India (Madhu and Madhu 2006); Tomini Bay in Sulawesi, Indonesia (Kuitert and Tonzuka 2004, cited by Timm *et al.* 2008); Cocos (Keeling) Islands and Christmas Island in the eastern Indian Ocean; Fiji; Micronesia; and as far north as the Ryukyu Islands in the East China Sea (Thornhill 2012). However, these reports are most likely cases of misidentification of *A. ocellaris* (Maarten De Brauwer, pers. comm. 2015), or indicative of a potential third, cryptic species in the species complex that has yet to be described.

Phylogenetic analysis from Timm *et al.* (2008) shows that specimens from Tomini Bay (Sulawesi, Indonesia) belong to the *A. percula* clade, but were genetically distinct from *A. percula* sampled within the known range of the species at the sub-clade level. These results suggest the possibility of a third, cryptic species present within the *A. ocellaris/percula* species complex, which is not concordant to the current taxonomy. More genetic analyses are required to determine whether the range of *A. percula* is more extensive than what has been described previously or if a third cryptic species does exist. The existence of a third species would not affect our analyses here as we have not considered the geographic area where this third species

might exist to be part of the range of *A. percula*. If further genetic research indicates there is no third species and the range of *A. percula* extends beyond what we have analyzed here, we could consider our conclusions to be conservative underestimates of range size and abundance. For the purposes of this analysis, we did not speculate as to which scenario is more likely; rather, we used the best available scientific and commercial information to determine the species' current range.

Overall, the area of coral reef habitat within the range of *A. percula*, based on satellite imagery and classification, is estimated between 36,000 km² and 50,000 km² (Burke *et al.* 2011; De Brauwier 2014 citing Fautin and Allen 1992 and Spalding *et al.* 2001). We are unable to provide an estimate of the non-coral reef habitat within the species' range including sandy patches between patch reefs, parts of lagoons, and seagrass beds. As such, the likely area where *A. percula* occurs is actually much greater and thus the estimated area is likely an underestimate.

Distribution and Connectivity

Distribution within Reef Habitats

The distribution of suitable host anemone species essentially dictates the distribution of *A. percula* within its habitat (Elliott and Mariscal 2001). Although competition with other anemonefish species may lead to finer-scale niche differentiation as previously described. *Heteractis crispa*, *H. magnifica*, and *S. gigantea* range throughout and beyond *A. percula*'s geographic extent. *Stichodactyla haddoni* occurs in Australia and Papua New Guinea, but has not yet officially been recorded in Vanuatu or the Solomon Islands, and *S. mertensii* officially has been recorded only from Australia within *A. percula*'s range (Fautin and Allen 1997; Fautin 2013). However, two recent observations extended the known distribution of *S. haddoni*, both northward and southward, indicating they have the ability to expand in range and facilitate the expanded occurrence of commensal species (Hobbs *et al.* 2014; Scott *et al.* 2014). Anecdotally, there are photo images and video footage of *S. haddoni* and *S. mertensii* in the Solomon Islands, Vanuatu, and Papua New Guinea (e.g., Shutterstock (**Vanuatu, Solomon Islands**), National Geographic (**Solomon Islands**), and Getty Images (**Papua New Guinea**)). Species experts, however, have not officially confirmed these reports.

Although geographically widespread, anemone species differ in their preferred habitat (e.g., reef zonation, substrate, depth (Fautin 1981)). Hattori (2006) found that *H. crispa* individuals were larger along reef edges and smaller in shallow inner reef flats. The larger anemones on reef edges experienced higher growth, probably because deeper (up to 4 m) reef edges provide more prey and lower levels of physiological stress. The author speculates that habitat and depth ideal for high anemone growth will vary by study site and occur at depths where there is a balance between available sunlight to allow for photosynthesis and low physiological stress, both of which are dependent on site-specific environmental conditions. Elliott and Mariscal (2001) found that coexistence of multiple clownfish species in close proximity is possible because of differences in their host anemone preference and differences in their habitat utilization. For example, in Madang, Papua New Guinea, both *A. percula* and *A. perideraion* primarily occupied *H. magnifica* hosts, but *A. percula* dominated *H. magnifica* in the nearshore zone while *A. perideraion* dominated in the offshore zone.

It is difficult to generalize the likely distribution, abundance, and trends of anemone hosts throughout the range of *A. percula*; these parameters are likely highly variable across the species' range. In an assessment done throughout the Great Barrier Reef, anemones, including those that host *A. percula*, were quantified as "common" (Roelofs and Silcock 2008). On the other hand, Jones *et al.* (2008) and De Brauwer *et al.* (in prep) note that anemones occur in relatively low densities throughout their range. Based on the available information, it is difficult to generalize the likely distribution of anemones, and therefore *A. percula* in coral reef environments throughout its range; it is likely to be variable and dependent on local environmental conditions. At least one study indicates that *A. percula* may prefer nearshore or shallower habitats (Elliott and Mariscal 2001).

Dispersal and Connectivity

Because orange clownfish are unable to migrate from one coral reef to another during their adult life stage, dispersal between reefs is limited to the species' approximately 8-12 day pelagic larval stage. There is support that simply having a pelagic larval phase can lead to some degree of long-range dispersal for species (Saenz-Agudelo *et al.* 2012; Treml *et al.* 2012). Pelagic larval duration is likely to be a strong predictor of dispersal distances (Shanks *et al.* 2003), although no direct evidence exists where both dispersal distance and pelagic larval duration have been measured. On the other hand, in a review of research on connectivity on coral reefs, Jones *et al.* (2009) conclude that variation in self-recruitment estimates within fish and coral species is more likely influenced by geographic isolation and spacing of reefs than pelagic larval duration, a conclusion supported by Pinsky *et al.* (2012). Williams and Hastings (2013) highlight the significance of temporally variable fluid dynamic processes leading to stochastic dispersal events for marine organisms.

Despite evidence correlating pelagic larval duration with dispersal distance, it has typically been a poor predictor of genetic similarity (Bay *et al.* 2006; Bowen *et al.* 2006; Weersing and Toonen 2009) and genetic population structure (Bay *et al.* 2006; Bowen *et al.* 2006; Luiz *et al.* 2012). Because small pelagic larvae are extremely difficult to track in the open ocean, population genetics is a useful technique to evaluate gene flow and connectivity between populations of coral reef associated organisms. Measuring self-recruitment rates in particular helps us to understand small scale connectivity in meta-populations of reef fish like *A. percula*. Using larval tagging, Almany *et al.* (2007) estimated that 60% of larvae they tagged around Kimbe Island, Papua New Guinea were spawned at Kimbe Island (self-recruiters) and the remaining 40% of tagged larvae were from reefs outside the study area, the closest of which is 10 km away. Using genetic parentage analysis, Planes *et al.* (2009) documented 42% self-recruitment for *A. percula* at Kimbe Island and found that the Kimbe Island population produced larvae that successfully dispersed to other populations 15-35 km away, revealing significant demographic connectivity. Based on these data (Planes *et al.* 2009), Buston *et al.* (2012) found the probability of successful dispersal between populations decreasing fivefold over 1 km of distance from a natal site. In yet another study, a total of 110 juvenile orange clownfish collected on Kimbe Island were screened, again using genetic parentage analysis; 46 were assigned to parents on Kimbe Island ("self-recruiters") and the remaining 64 were classified as "immigrants" that arrived from another reef at least 10 km away (Berumen *et al.* 2010). Overall, while *A. percula* dispersal between reef groups in Kimbe Bay appears variable over time (Berumen *et al.* 2012), the above studies' findings consistently indicate that self-recruitment around Kimbe Island ranges from 42-60%,

and there exists significant connectivity at the scale of 10s of km for *A. percula*. Anemonefish larvae possess significant locomotory and sensory abilities that may help them avoid dispersal away from the sensory halo of Kimbe Island (Fisher *et al.* 2000; Dixon *et al.* 2008); however, other factors, presumably including local current patterns, must also influence larval dispersal, to some degree (Berumen *et al.* 2012). While *A. percula* populations in Kimbe Bay have been well studied, it is unclear how representative these results may be of other areas within the species' range. In addition to the density of *A. percula* likely being highest in Kimbe Bay and nearby areas, aspects of the geographical setting that influence dispersal will also vary among sites across the species' range, leading to variable levels of self-recruitment.

Research on congeners of *A. percula* has revealed similar variability in levels of self-recruitment at different locations. For example, along 28 km of coastline near Port Moresby, Papua New Guinea, study results showed highly variable levels of self-recruitment of *A. polymnus* among sites, but overall low observed self-recruitment rates, a high proportion of connectivity among sites, and a relatively high proportion of long-distance dispersal. This led to the conclusion that connectivity, not self-recruitment, dominates larval replenishment in this focal clownfish meta-population (Saenz-Agudelo *et al.* 2011). The authors noted that differences in self-recruitment rates among sites within the meta-population were likely linked to different localized geographies that affect access and proximity to alongshore current flows (Saenz-Agudelo *et al.* 2011). In contrast, Jones *et al.* (2005) found high levels of self-recruitment for *A. polymnus* at sites within Kimbe Bay, which supports the notion that the level of self-recruitment in a meta-population likely depends on the level of geographic isolation. Another congener, *A. melanopus*, was included in a list of species studied on the Great Barrier Reef and found to have generally high genetic diversity that did not differ significantly among species and locations (Bay *et al.* 2006). Additionally, genetic parentage analysis of *A. bicintus* in the Red Sea found minimal to no self-recruitment in two consecutive years, indicating a virtually open population over several kilometers (Nanninga 2013). Moreover, van der Meer *et al.* (2012) report 68-84% self-recruitment at three island marine protected areas in Australia for the endemic *A. mccullochi*, while self-recruitment for *A. ocellaris* and *A. perideraion* ranged from 44-65% among sites and sampling periods in the Spermonde Archipelago, Indonesia (Madduppa *et al.* 2014). The variability in self-recruitment rates among these species and populations is likely to be driven by the variable nature of the geographical settings of the different study locations. In well-connected populations, self-recruits are diffused by immigrants; the opposite is true for geographically isolated populations that tend to show higher rates of self-recruitment.

On a larger geographic scale, Treml *et al.* (2012), hypothesize that broad-scale connectivity is strongly influenced by reproductive output and length of pelagic larval duration based on a biophysical model of larval dispersal. However, there are limitations to this approach when considering species that actively swim during their pelagic larval phase, like some anemonefish, rather than passively disperse. This is evidenced by the fact that, for several clownfish species, there have been conflicting results regarding whether regional ocean currents are a good predictor of phylogeographic structure and, therefore, whether such passive dispersal modeling based on ocean currents is informative. For example, Nelson *et al.* (2000) mapped the phylogeography of *A. ocellaris* across its range and found that surface ocean currents in the region, which should influence larval dispersal, were poorly correlated with phylogeographic structure. Instead, *A. ocellaris* phylogeography is more reminiscent of Pleistocene sea level changes and flooding of the Sunda shelf. Omani clownfish populations on the other hand,

separated by over 400 km, were found to be connected in both directions using genetic assignment tests; this long-distance dispersal matched predictions from a dispersal model based on oceanographic currents, demonstrating that simple physically forced models can give valuable predictions for realized patterns of connectivity in some cases (Simpson *et al.* 2014).

In the absence of a broad-scale phylogeographic study for *A. percula*, we are left with small-scale meta-population connectivity studies as the best available information. Results from studies in Kimbe Bay, Papua New Guinea, indicate that *A. percula* have the ability to disperse at least up to 35 km away from natal areas (Planes *et al.* 2009). In addition, there is evidence that rates of self-recruitment are likely to be linked with not only pelagic larval duration, but also geographical isolation (Jones *et al.* 2009; Pinsky *et al.* 2012). Because of the size and distribution of *A. percula*'s range, there are likely areas of higher and lower connectivity throughout, linked with the variability in geographic isolation across locations, creating significant spatial structure. This is, however, speculative because no large-scale connectivity study has been conducted for this species.

Based on the best available information, we consider that the species is likely to have highly variable small scale connectivity among and between meta-populations, but unknown large scale genetic structure across its entire range.

Estimated Abundance

Based on the best available information, densities of meta-populations of *A. percula* are highly variable throughout the species' range. Densities are highest in the northern region of Papua New Guinea, which has the greatest reported species diversity and densities of both anemonefishes and anemones (Elliott and Mariscal 2001). In Madang, Papua New Guinea, Elliott and Mariscal (2001) estimated the total density of *A. percula* at approximately 0.79 fish per 100 m², or approximately 7,900 individuals per km² of reef area. In Kimbe Bay, Papua New Guinea, Planes *et al.* (2009) counted 975 individuals (506 adults and 469 juveniles) in 270 anemones in a 1 km² area of reef. Density of *A. percula* is lower at survey locations in the Solomon Islands and Australia than Papua New Guinea sites (Maarten De Brauwer, pers. comm. 2015). De Brauwer (2014) determined an average density for the species within its range from 658 surveys across 205 sites throughout the species' range. He calculated the global estimated mean density at 0.09 fish per 250 m², or 360 fish per km².

With no existing estimate of global abundance for *A. percula*, we estimated, based on the best available information a total of 13-18 million individuals for the species throughout its range. Acknowledging that density estimates for *A. percula* are highly variable throughout the species' range, we used De Brauwer's (2014) estimated average density of 360 fish per km² to derive global abundance. In order to extrapolate this average density to estimate abundance, we used two different estimates of coral reef area within the species' range. De Brauwer (2014) estimated 36,000 km² of coral reef area within the species' range based on Fautin and Allen (1992, 1997) and Spalding *et al.* (2001). We also used newer coral reef mapping data from Burke *et al.* (2011; ArcGIS shapefiles of coral reef area provided by K. Reytar) resulting in an estimate of approximately 50,000 km² of coral reef area within *A. percula*'s range. We used both values to determine a range of estimated abundance (13-18 million) to reflect uncertainty. It is important to note that this may be an underestimate because it is based on coral reef area, which likely does not account for most of the non-reef area where the species occurs throughout its

range. We found no information on historic abundance or recent population trends for *A. percula* throughout all or part of its range.

ASSESSMENT OF EXTINCTION RISK

Approach to Evaluating Extinction Risk

In evaluating extinction risk to the species, we identified the four demographic risk factors suggested by Wainwright and Kope (1999) and McElhany *et al.* (2000), as well as the threat factors identified in section 4 of the ESA. The four demographic viability risk criteria, considered at the species level include: abundance, growth rate/productivity, spatial structure/connectivity, and diversity. These viability criteria reflect concepts that are well founded in conservation biology and that individually and collectively provide strong indicators of extinction risk. The approach of considering demographic risk factors to help frame the consideration of extinction risk has been used in many status reviews including Pacific salmonids, Pacific hake, walleye pollock, Pacific cod, Puget Sound rockfishes, Pacific herring, scalloped hammerhead sharks and black abalone.² In addition to these factors, we considered the five threat factors listed in section 4(a)(1) of the ESA. Based on all of this information, we describe the likely level of extinction risk faced by the orange clownfish both now and in the foreseeable future (defined below).

Because information on the orange clownfish is sparse and often non-quantitative, we used qualitative risk categories to characterize the likelihoods of the four demographic viability criteria significantly contributing to extinction risk: very low, low, medium, high or unknown. In addition, because some threats to the orange clownfish are either poorly understood or unquantifiable, we characterized the relative likelihood (as very low, low, medium, high, or unknown) that threats (confirmed, potential or assumed) are significantly contributing to extinction risk for *A. percula*. We do not make recommendations as to whether the species should be listed as threatened or endangered. Rather, conclusions are drawn about the overall risk of extinction faced by the species under present conditions and in the foreseeable future based on an evaluation of the species' demographic risks and threats.

Although the orange clownfish is not officially listed under the IUCN Red List of Threatened Species, De Brauwer (2014) assessed extinction risk for all anemonefish species using the IUCN Red List criteria. De Brauwer used multiple methods to calculate values for each criterion (18 methods in total to calculate different values for the 4 criteria) and *A. percula* was most commonly assigned to the "Least Concern" category. The Least Concern category is used to highlight species that have a relatively low extinction risk compared with those taxa that are assessed as Near Threatened, Threatened, Endangered or Critically Endangered. For De Brauwer (2014), the only scenario where *A. percula* was conservatively assigned to a higher IUCN risk category ("Endangered") resulted from one of the methods he used to calculate the area of occupancy. Using this most conservative method to determine area of occupancy to

² See <http://www.nmfs.noaa.gov/pr/species/> for links to these reviews.

assess risk of extinction for all anemonefish, 20 species could potentially be classified as Endangered and 8 species as Critically Endangered (De Brauwer 2014). Although IUCN Red List criteria differ from how we assess species under the ESA, it is a useful exercise to inform our analysis of *A. percula*'s risk of extinction. Risk classifications by other organizations, or made under other Federal or state statutes, may be informative, but the classification alone may not provide the rationale for evaluating species status under the ESA. Rather, we evaluate the sources of information on which the classification is based and determine how this information informs our assessment of extinction risk and whether a species meets the definition of threatened or endangered under the ESA.

According to the ESA, the determination of whether a species is threatened or endangered should be made on the basis of the best scientific information available regarding its current status, after taking into account efforts being made to protect the species. During the extinction risk assessment, likely or possible effects of conservation measures are taken into account to the extent they are reflected in metrics of population or species viability. Conservation measures that have not yet been implemented or shown to be effective are taken into account in a separate process by NMFS prior to proposing any listing determinations. In the last part of this section, we summarize the conservation efforts that are currently in place that may be benefiting the orange clownfish so that NMFS can consider them before making a listing determination.

Foreseeable Future

The term “foreseeable future” is not defined in the ESA. The NMFS guidance for conducting status reviews under the ESA instructs us to interpret this phrase as the timeframe over which predictions about the future conservation status of the species can be reasonably relied upon (NMFS 2013). Those predictions can be in the form of extrapolation of population or threat trends, analysis of how threats will affect the status of the species, or assessment of future events that will have a significant new impact on the species. We consider the life history of the species, habitat characteristics, availability of data, kinds of threats, ability to predict threats, and the reliability of models used to forecast threats over that “foreseeable future” in determining the time period that constitutes the foreseeable future. This approach does not limit the time frame under consideration to the length of time into the future for which a species’ status can be quantitatively modeled or predicted within predetermined limits of statistical confidence, although uncertainties of any modeling efforts should be documented. Because a species may be susceptible to a variety of threats for which different data are available or which operate across different time scales, the foreseeable future is not necessarily reducible to a particular number of years. Thus, we may describe the foreseeable future in general or qualitative terms.

In our consideration of the foreseeable future for this status review, we evaluated how far into the future we could reliably predict the operation of the major threats to this species, as well as the species’ response to those threats. We are reasonably confident in our ability to predict out several decades in assessing all threats listed below and their interaction with the life history of the orange clownfish. We considered generation length to reflect turnover of breeding individuals and account for non-breeding older individuals. Estimates for generation length, which is greater than first age of breeding but lower than the oldest breeding individual (IUCN 2015), of the orange clownfish range between 6 and 15 years. Therefore, we conservatively define the foreseeable future as 30 years from the present, which is based on 2-3 generation

lengths as well as the estimated lifespan of the orange clownfish. We note that, while we may have information with which to predict the trajectory of a particular threat into the future for a defined amount of time (e.g., climate projections out to the year 2100), we may lack information on the species' response to that threat over the same time period, and therefore do not extend the foreseeable future as far as we can predict the trajectory of such a threat.

Demographic Risks

Summarized below are various demographic risks to the viability of *A. percula*. As mentioned previously, we conducted a qualitative assessment of the overall status of the species using four demographic viability risk criteria: abundance, population growth rate/productivity, spatial structure/connectivity, and diversity. The relative likelihood that each particular demographic factor contributes, or will contribute in the foreseeable future as defined above, in a significant way to extinction risk of the species is summarized at the end of this section (Table 1) according to the following scale:

- (1) Very low – it is very unlikely that the particular factor contributes or will contribute significantly to risk of extinction;
 - (2) Low – it is unlikely that the particular factor contributes or will contribute significantly to risk of extinction;
 - (3) Medium – it is likely the particular factor contributes or will contribute significantly to risk of extinction; and,
 - (4) High – it is highly likely that the particular factor contributes or will contribute significantly to risk of extinction.
 - (5) Unknown – it is unknown how the particular factor contributes or will contribute to risk of extinction.
- (Note: The term “significantly” is used here as it is generally defined – i.e., in a sufficiently great or important way as to be worthy of attention.)

If there was some uncertainty between two of the qualitative categories for any particular demographic factor, and/or uncertainty in the available information for that factor, we conservatively assigned it to the higher level of likelihood out of the two.

Abundance

In general, very low levels of a species' abundance and density may cause difficulty in mate choice; sex-ratios; fertilization and recruitment success; reproductive or courting behaviors; foraging success; and predator avoidance behaviors. There are no global historical or current abundance estimates for *A. percula* in the literature. However, we estimate global, wild population abundance for *A. percula* to be approximately 13-18 million individuals based on De Brauwer (2014) and estimates of total habitat area. We believe this estimate to be a conservative estimate for reasons discussed in section 2.9 above.

Based on our estimate of global abundance and information gleaned from the literature on the reproductive strategy of the species, we determined that the species' abundance is not so low that it is at risk of extinction due to environmental variation, anthropogenic perturbations or demographic stochasticity. *Amphiprion percula*'s reproductive strategy is not dependent on finding a mate or affected by sex ratios, so compensatory processes are not of great concern. An

abundance of 13-18 million individuals is sufficiently high to provide genetic diversity, which is supported by results from several studies of connectivity for the species (Almany *et al.* 2007; Planes *et al.* 2009; Berumen *et al.* 2012; Buston *et al.* 2012). We found no evidence that the species is not fulfilling its ecological role due to low abundance in any part of its range. While we acknowledge that there is uncertainty associated with our estimate of population abundance, it was determined based on the best available information (De Brauwer 2014) and is deemed conservative as it does not encompass areas such as seagrass beds known to harbor significant numbers of anemones with anemonefish, especially in Papua New Guinea (Colette Wabnitz, pers. comm. 2015). Overall, the best available information indicates that the estimated current global, wild abundance of *A. percula* is unlikely to be contributing to the extinction risk of the species.

Population Growth Rate / Productivity

Amphiprion percula are estimated to have a minimum population doubling time of less than 15 months (Florida Museum of Natural History 2005; Fishbase.org 2015), and low vulnerability to fishing based on biological and life history parameters (Cheung *et al.* 2005; Fishbase.org 2015). Natural mortality for juveniles and adults is low, ranging from 2% (Elliott and Mariscal 2001) to ~7% for ranks 1-3 and ~30% for ranks 4-6 (Buston 2003a). The species is also highly productive and can spawn monthly, with an individual laying from 100 to over 1000 eggs during each spawning session, depending on fish size and previous experience (Fautin and Allen 1997). There are currently no data that provide an estimate of how long orange clownfish spawn during their life span.

We do not know the recent or historical trend of the global population of orange clownfish and therefore cannot estimate an overall population growth rate or decline. However, their high fecundity in combination with low juvenile and adult mortality and estimated minimum population doubling time of 15 months all indicate that populations are likely to be resilient to periodic disturbances. It is important to note that the suitable number of host anemones, not availability of new anemonefish recruits, is what limits anemonefish populations (Fautin and Allen 1997). This notion is supported by the observation in Madang Lagoon, Papua New Guinea in 1997 that when non-breeders were removed they were rapidly replaced (Buston 2003c), indicating that the population was basically at carrying capacity. We recognize that, based on this limitation, the population growth rate and status of *A. percula* is very closely linked to the population status of its host anemone species. However, because data on anemones are virtually non-existent, it is difficult to generalize the likely distribution, abundance, and trends of *A. percula*'s anemone hosts, as described above in section 2.8. We use the best available information to assess threats to host anemones below in section 3.3.1. Based on the conclusion below in section 3.3.1 in combination with the best available information presented here, we determined that population growth rate/productivity of *A. percula* is unlikely contributing to extinction risk for the species.

Spatial Structure / Connectivity

We can consider spatial structure for *A. percula* on different scales and have variable amounts of evidence to inform each level of assessment. As described above, multiple DNA parentage analyses have been conducted in and around Kimbe Bay, Papua New Guinea, that offer some insight into meta-population connectivity for this species in those locations. Self-recruitment

rates range from 40% to over 60% (Almany *et al.* 2007; Planes *et al.* 2009; Buston *et al.* 2012). In one study, up to 10% of *A. percula* larvae were long distance migrants that successfully recruited to reefs up to 35 km away (Planes *et al.* 2009). This was originally the longest direct measure of larval dispersal distance for any marine fish species reported using the genetic parentage methodology until Simpson *et al.* (2014) reported Omani clownfish (*A. omanensis*) migrating over 400 km from their natal origins. It also demonstrates significant demographic connectivity within Kimbe Bay. These results are also consistent with more general biophysical modeling for the tropical western Pacific region, which suggests high levels of connectivity in regions where reefs are only 20-30 km apart, including for species with a wide range of pelagic larval durations (Treml *et al.* 2008). Connectivity information for congeners that inhabit the same areas and habitat types support the variability reported in self-recruitment and high connectivity of meta-populations (Bay *et al.* 2006; Saenz-Agudelo *et al.* 2011; van der Meer *et al.* 2012; Nanninga 2013; Madduppa *et al.* 2014; and Saenz-Agudelo *et al.* 2015). As reported earlier, connectivity and self-recruitment rates for meta-populations are likely based on local geographic settings (Jones *et al.* 2009) and environmental conditions, which explains the variability between study sites in different locations.

On a broader geographic scale, Timm *et al.* (2008) report that the close relatedness of genetic samples of *A. percula* from sites in New Britain (Papua New Guinea) and the Solomon Islands could indicate connectivity in a southeast direction, at least historically. However, it remains unclear how closely related individuals from various parts of the species' range are on a broad scale currently; there may be some dispersal barriers within the range of *A. percula* of variable strength (Treml *et al.* 2015). We have insufficient genetic information to identify whether critical source populations exist; this epistemic uncertainty can only be reduced through further research.

Amphiprion percula's spatial structure appears to consist of a series of meta-populations throughout the species' range that have variable rates of both internal and external connectivity likely linked to varying degrees of geographic isolation. The best available information indicates that at a local scale, *A. percula* meta-populations experience variable levels of self-recruitment related to geographic isolation but can disperse up to 35 km away from their origins. Globally, however, there is no information available on the overall spatial and genetic structure for the species. Therefore, we conclude that the level to which spatial structure and connectivity is likely to be a factor contributing to extinction risk for *A. percula* is unknown.

Diversity

The loss of diversity can reduce a species' reproductive fitness, fecundity, and survival, thereby contributing to declines in abundance and population growth rate and increasing extinction risk (e.g., Gilpin and Soulé 1986). There is some uncertainty, however, as to whether the loss of diversity by itself confers risk of extinction (see Brook *et al.* 2002). Although the loss of diversity certainly increases extinction risk through its compounding effects on other demographic factors, it is argued by some that the loss of diversity by itself plays a relatively minor role in extinctions. The loss of diversity can help bring species to a high risk status, but other demographic or environmental factors usually play the direct role in causing extinctions (Lande 1988; Caro and Laurenson 1994; Caughley 1994; Dobson 1999).

For *A. percula*, we found no evidence that genetic diversity is a factor limiting the success of the species and contributing to extinction risk. Meta-populations of this species exhibit variable levels of connectivity that appear sufficient to maintain genetic diversity. Heterogeneity of habitats across the species' range also likely leads to behavioral or physiological adaptations to local environmental conditions, thereby increasing diversity. Examples may be preference for one anemone species over another, preference for anemones that are nearshore versus offshore, or other local behavioral and/or physiological adaptations. In addition, a mixture of genetic and environmental factors usually causes phenotypic diversity; clownfish species often exhibit geographical color morphs or other phenotypic diversity (Moyer 1976). Militz (2015) reports a broad diversity in color forms for *A. percula* in Kavieng, New Ireland, Papua New Guinea including variation in the width of the black border along its white bars (also observed by Timm *et al.* 2008) or "mis-barred" individuals that have some variation in vertical white bars from the norm for the species. Based on genetic and phenotypic diversity exhibited by the species, we conclude that genetic diversity is unlikely to be contributing to extinction risk for *A. percula*. Based on the best available information/data, we assigned a low likelihood to three of the four demographic risk factors because we determined they are unlikely to contribute significantly to extinction risk for *A. percula* both now and in the foreseeable future. On a local scale, spatial structure and connectivity do not appear to be a cause for concern for this species but, because global genetic structure is unknown, we cannot assign a likelihood that this factor is contributing to extinction risk for *A. percula*.

Analysis of the ESA Section 4(A)(1) Factors

According to section 4 of the ESA, the Secretary determines whether a species is threatened or endangered as a result of any of the following five factors: (A) Present or threatened destruction, modification, or curtailment of habitat or range; (B) overutilization; (C) disease or predation; (D) inadequacy of existing regulatory mechanisms; or (E) other natural or manmade factors. Collectively, these five factors are referred to here as "threats." The following sections provide information on the threats from each of these five factors as they relate to the status of the orange clownfish. The likelihood that each particular threat is contributing to extinction risk for the orange clownfish is summarized at the end of this section (Table 2) according to a qualitative scale:

- (1) Very low – it is very unlikely that the particular threat contributes or will contribute significantly to risk of extinction;
- (2) Low – it is unlikely that the particular threat contributes or will contribute significantly to risk of extinction;
- (3) Medium – it is likely the particular threat contributes or will contribute significantly to risk of extinction; and,
- (4) High – it is highly likely that the particular threat contributes or will contribute significantly to risk of extinction.
- (5) Unknown – it is unknown how the particular factor contributes or will contribute to risk of extinction.

(Note: The term "significantly" is used here as it is generally defined, the same way it is used under section 3.2 – i.e., in a sufficiently great or important way as to be worthy of attention.)

If there was some uncertainty between two of the qualitative categories for any particular threat and/or uncertainty in the future projections or impacts of the threat, we conservatively assigned it to the higher level of concern.

Factor A: Present or Threatened Destruction, Modification or Curtailment of Habitat or Range

Anemone Bleaching

Amphiprion percula is described as a habitat specialist due to its symbiotic association primarily with three species of anemone: *Heteractis crispa*, *H. magnifica*, and *Stichodactyla gigantea* (Ollerton *et al.* 2007), although the species has also been reported associating with *S. mertensii* (Elliott and Mariscal 2001) and *S. haddoni* (Planes *et al.* 2009). As habitat specialists, the symbiotic relationship between *A. percula* and its host anemone makes this species indirectly susceptible to threats that are likely to impact its host anemone. Anemones and their symbiotic anemonefish inhabit coral reefs and nearby habitats such as lagoons, seagrass beds, and sand flats (Fautin and Allen 1997). NMFS recently reviewed and summarized the best available scientific information on the effects of climate change on corals and coral reefs in the final rule listing 20 coral species as threatened (79 FR 53851).

In summary, increased ocean temperatures are already affecting coral reef communities worldwide and will continue to do so. While all model results provide evidence that coral reef communities will be negatively affected in the future, the *magnitude* of those effects varies widely over both spatial and temporal scales. Coral reefs and other nearshore areas are highly heterogeneous habitats that experience yearly, seasonal, daily, and even more frequent fluctuations in environmental conditions. While some models suggest disastrous effects of climate change on coral reefs by the year 2100, such projections are based on spatially coarse analyses associated with high uncertainty, especially at local spatial scales. In addition, the majority of relevant studies use models based on temperature anomalies and thresholds only; however, McClanahan *et al.* (2015) found that temperature threshold indices had weak or no significant relationship with observed coral cover and susceptibility before, during, and after a major bleaching event. A more comprehensive multiple parameter approach showed significant fit with those field observations, indicating heterogeneous environmental causes and responses to climate disturbances and warming over space and time. Determining the effects of global threats on an individual coral species over the foreseeable future was described in the coral final listing rule as complicated by the combination of (1) uncertainty associated with projected ocean warming and acidification threats; (2) regional and local variability in global threats; (3) large distributions and high habitat heterogeneity of the species; and (4) limited species-specific information on responses to global threats. These complicating factors also apply to determining the effects of future climate change on anemone species that host *A. percula*. The limited available information for anemones is described below.

Just like corals, anemones have symbiotic relationships with microscopic algae known as zooxanthellae. Because of this symbiosis, they are susceptible to thermally-induced bleaching, during which those symbionts are expelled due to thermal stress (Hill and Scott 2012). Anemone vulnerability to bleaching events is dependent on susceptibility, exposure, and adaptive capacity

or response, each of which are influenced by a host of biological and environmental factors, differ across species and locations, and can change over time (Hobbs *et al.* 2013).

Bleaching events may affect host anemone species by causing reductions in abundance of anemone populations and/or a reduction in size of bleached anemones (Hattori 2002; Saenz-Agudelo *et al.* 2011; Hill and Scott 2012). The degree of impact of such bleaching events varies widely. Following a mass bleaching event off Sesoko Island, Japan, the number of observed *H. crispa* hosts decreased by over 80% (Hattori 2002). In comparison, near Port Moresby, Papua New Guinea, a bleaching event resulted in the loss of 3 out of 55 *S. haddoni* and *H. crispa* anemones and a reduction in size of the bleached anemones by approximately 34% (Saenz-Agudelo *et al.* 2011). While reductions in size were evident in shallower waters at the Papua New Guinea site, anemones found at depths >7 m were not affected by the bleaching and researchers noted that all remaining impacted anemones recovered full pigmentation two years after the bleaching event (Saenz-Agudelo *et al.* 2011). Variable impacts to anemonefish assemblages in response to anemone bleaching events have also been reported. These range from the local extinction of one species of *Amphiprion* due to displacement by another *Amphiprion* species at one site (Hattori 2002), to only short-term changes in female egg production and recruitment at a different site (Saenz-Agudelo *et al.* 2011).

The evidence described above, while limited, indicates that thermally-induced bleaching can have negative impacts on orange clownfish host anemones, which may lead to localized impacts of unknown magnitude on the fish itself. Evidence thus far indicates high variability in the response of both anemones and anemonefish to localized bleaching events. As noted above, susceptibility to thermal stress varies between different species of the same taxon and is often variable within individual species; as a result of habitat heterogeneity across a species' range, individuals of the same species may develop in very different environmental conditions. Hobbs *et al.* (2013) compiled datasets that were collected between 2005 and 2012 across 276 sites at 19 locations in the Pacific Ocean, Indian Ocean, and Red Sea to examine taxonomic, spatial, and temporal patterns of anemone bleaching. Their results confirm that bleaching has been observed in 7 of the 10 anemone species that host anemonefish (including four of the five *A. percula* host species), with anecdotal reports of bleaching in the remaining 3 host anemone species. In addition, they report anemone bleaching at 10 of 19 survey locations that are geographically widespread. Importantly, they report considerable spatial and inter-specific variation in bleaching susceptibility across multiple major bleaching events (Hobbs *et al.* 2013). Over the entire timeframe and across all study areas, 3.5% of all anemones observed were bleached, although during major bleaching events, the percentage at a given study area ranged from 19-100%. At sites within the same study area, bleaching ranged between as much as 0 and 94% during a single bleaching event. To further highlight the variability and uncertainty associated with anemone bleaching susceptibility, Hobbs *et al.* (2013) report opposite patterns of susceptibility for the same two species at the same site during two different bleaching events. Additionally, the study reports decreased bleaching with increased depth in most of the major bleaching events, indicating that depth, in some cases as shallow as 7 m, offers a refuge from bleaching (Hobbs *et al.* 2013). Some anemone species have even been reported from mesophotic depths, including one *A. percula* host species (*H. crispa*) (Bridge *et al.* 2012). These depths likely serve as refugia from thermal stress. Although the capacity for acclimation or adaptation in anemones is unknown, evidence from one site indicated that prior bleaching history may influence subsequent likelihood of an anemone bleaching, as previously bleached individuals

were less likely to bleach a second time (Hobbs *et al.* 2013). It is also of note that, similar to corals, bleaching does not automatically lead to mortality for anemones. Hobbs *et al.* (2013) report variable consequences as a result of bleaching between and among species and locations in their assessment of bleaching for all anemone species that host anemonefish (including those that host *A. percula*); some species decreased in abundance and/or size after bleaching events, while others showed no effect and recovered fully.

When considering the effect of anemone bleaching into the foreseeable future, we evaluated the best available information on future projections of warming-induced bleaching events, but also considered the existing information on the impacts of previous bleaching events on anemones. Evidence suggests that bleaching events will continue to occur and become more severe and more frequent over the next few decades (van Hooidonk 2013). However, newer multivariate modeling approaches indicate that traditional temperature threshold models may not give an accurate picture of the likely outcomes of climate change for coral reefs, and impacts and responses will be highly nuanced and heterogeneous across space and time (McClanahan *et al.* 2015). Although observed anemone bleaching has thus far been highly variable during localized events, the overall effect of bleaching events on anemones globally (i.e., overall proportion of observed anemones that have shown ill effects) has been of low magnitude at sites across their ranges, as only 3.5% of the nearly 14,000 observed anemones were recorded as bleached across 19 study sites and multiple major bleaching events (Hobbs *et al.* 2013). The low overall effect thus far, high amount of variability in anemone susceptibility, existence of depth refugia for anemones, evidence of potential acclimation in some species, and the fact that *A. percula* has been observed in the wild to associate with at least five different species of anemone that have shown different levels of susceptibility to bleaching in different locations and over time, are all factors that, in combination, indicate that *A. percula* is likely resilient to bleaching impacts that may affect their hosts both now and in the foreseeable future. As such, we conclude that the threat of habitat loss due to anemone bleaching has a low likelihood of contributing to extinction risk for *A. percula* now or in the foreseeable future.

Anemone Collection

Just like the fish they host, anemones are often collected for the marine aquarium trade. There has been a recent shift in home aquaria from fish-only tanks to tanks that recreate mini-reef environments with the inclusion of live invertebrates and corals, as well as live rock along with tropical reef fish (Murray and Watson 2014). In a survey of 314 home aquarium hobbyists, 39% indicated they have anemones in their tanks (Murray and Watson 2014). Thus far, there has been limited successful aquaculture of anemones for aquaria; Moe (2003) reports the results from a survey of hobbyists, scientists, and commercial breeders indicating several species have been successfully propagated (typically via asexual reproduction), but anemones typically thwart both scientific and hobbyist attempts at captive culture, especially on a large scale. As such, the vast majority of anemone specimens in the trade are currently from wild collection. There is little information available on the amount of collection or trade in wild anemones; none are listed under the Convention of International Trade in Endangered Species of Wild Flora and Fauna (CITES), so import/export of these species does not require CITES documentation, which otherwise might be a source of information about the magnitude of trade.

Limited information is available on the impacts of collection to anemone populations in Australia. Jones *et al.* (2008) surveyed two different regions of the Great Barrier Reef with

contrasting disturbance histories to determine the degree to which densities of anemones and anemonefish are driven by bleaching and collection. They found that collection has likely exacerbated the effects of bleaching and other disturbances on anemones in the Keppel Islands region. In contrast, high densities of anemones and anemonefish were recorded in the Far North Queensland region where collection also occurs, but fewer other disturbances have occurred. The authors note that the Keppel Islands region is isolated from other reef areas, which likely affects its ability to recover from disturbances via input from nearby reefs. *Amphiprion percula* does not occur as far south as the Keppel Islands region and is therefore not experiencing the declines in anemone hosts reported for this area. The species does occur within the Far North Queensland region, which has experienced fewer disturbances and shows higher densities of both anemones and anemonefish. Based on a combination of criteria including accessibility, habitat/ecological niche, distribution, and abundance, Roelofs and Silcock (2008) found that all anemone species had low vulnerability due to collection in the Queensland fishery; however, similar information is not available for other parts of *A. percula*'s range. While not in *A. percula*'s range, Shuman *et al.* (2005) surveyed reefs and obtained catch records from marine ornamental collectors over a four-month period in the vicinity of Cebu, Philippines. Data showed that anemonefish and anemones comprised close to 60% of the total catch, and collection of anemones reduced the density of anemonefish at those sites by over 80% compared to non-fished areas. While there was no information on anemone collection available from the Solomon Islands, Vanuatu, or Papua New Guinea (likely because these countries tend to focus on exporting fish vs. invertebrates), our assessment reveals that collection and export of aquarium reef species, including anemones, in these three countries is relatively small-scale at just a few sites scattered throughout large archipelagos. The industry appears limited by freight costs and other financial burdens, and in each country exports leave from the largest cities (Kinch 2008). As such, it seems unlikely that collection would expand to other areas within the species' range and have more success.

In summary, although there is little information available on the threat of anemone collection to *A. percula* globally, the aquarium trade collection information from countries within the species' range indicates that fisheries in general are relatively small scale, and tend to focus on fish rather than invertebrates for export. As such, we do not deem this threat as a cause for concern for this species. There is no information to indicate that demand for wild harvested anemones will increase over the next few decades within the range of *A. percula*; although speculative, scientists and hobbyists are likely to continue to engage in attempts to propagate anemones in captivity, which may lead to lower demand for wild capture if successful. Because there is some uncertainty and a lack of specific information associated with this threat to *A. percula*, we conclude that the threat of habitat loss from anemone collection poses a low (instead of very low) likelihood of contributing to extinction risk for *A. percula*, both now and in the foreseeable future.

Sedimentation and Nutrient Enrichment

Localized impacts to coral reef habitat from land-based sources of pollution causing increases in sedimentation and nutrient enrichment are another potential source of habitat alteration that could affect *A. percula* and its anemone hosts. To date, efforts to examine the direct and indirect effects of nutrients and sedimentation to the orange clownfish throughout its range are lacking. However, we can provide some general information on the impacts of sedimentation and nutrient

enrichment on the coral reef habitats where *A. percula* occurs. Elevated sediment levels are generated by poor and/or destructive land use practices (e.g., slash and burn, logging) and coastal and nearshore construction. Sediments are then introduced into the ocean by a variety of mechanisms, including river discharge, surface runoff, groundwater seeps, and atmospheric deposition. The main vectors of anthropogenic nutrients are point-source discharges (such as rivers, treatment plants, septic leakage, or sewage outfalls) and surface runoff from modified watersheds. Natural processes, such as *in situ* nitrogen fixation and delivery of nutrient-rich deep water by internal waves and upwelling, also bring nutrients to coral reefs. Exposure to both sedimentation and nutrients is expected to increase with further expansion of human settlement along coastal margins and activities that generate sediment and nutrients.

While information for anemones is sparse, we know that some coral species can tolerate complete burial in sediment for several days; however, those that are unsuccessful at removing sediment may be smothered, resulting in mortality (Nugues and Roberts 2003). Sediment can also induce sub-lethal effects in corals, such as reductions in tissue thickness, polyp swelling, zooxanthellae loss, and excess mucus production (Rogers 1990). In addition, suspended sediment can reduce the amount of light in the water column, making less energy available for photosynthesis and growth. Again for corals, sedimentation and nutrient enrichment can have interactive effects with other stressors including disease and climate factors such as bleaching susceptibility and reduced calcification (Ateweberhan *et al.* 2013; Suggett *et al.* 2013). Wiedenmann *et al.* (2013) found that unfavorable ratios of dissolved inorganic nutrients in the water column led to phosphate starvation of symbiotic algae in corals, reducing their thermal tolerance. Cunning and Baker (2013) found higher nutrient loads can lead to higher densities of symbionts, and corals with higher densities of symbionts were more susceptible to bleaching. There is very little information available regarding the susceptibility and exposure of anemones to sedimentation and nutrients. In the absence of this information, we consider it reasonable to assume that the susceptibility of corals as a direct result of their association with symbiotic algae (described above) is an indicator of the potential susceptibility of anemones, since they share a similar association with microscopic algal symbionts. Exposure of host anemones is likely to be variable across the range of *A. percula*, with impacts being more acute in areas of high coastal development.

In addition to the potential impacts to host anemones, Wenger *et al.* (2014) found in a controlled experiment that suspended sediment increased pelagic larval duration for *A. percula*. A longer pelagic larval duration may reduce the number of larvae that make it to the settlement stage because of the high rate of mortality during this phase. Conversely, in this study longer pelagic larval durations led to larvae that were larger with better body condition, traits that may confer advantages during the first few days of settlement when mortality is still high for those that do recruit to settlement habitat. As such, the overall impact of increased sedimentation at the population level is hard to predict.

Distance is less of a moderating factor for nutrients than for sedimentation. Exposure to sedimentation can be moderated by distance of some habitats from areas where these impacts are chronically or sporadically heavy, resulting in some habitats being unaffected or very lightly affected by sedimentation. However, nutrient enrichment can still result from inputs from even sparsely populated areas, and these nutrients can be quickly transported large distances.

Similarly, although the depth of some habitats may also moderate nutrient exposure, nutrient impacts tend to extend deeper than sedimentation impacts.

Land-based sources of pollution are of primary concern for nearshore marine habitats in areas where human populations live in coastal areas and engage in any or all of the following: intensive farming and aquaculture, urbanization and industrialization, greater shipping traffic and fishing effort, and deforestation and nearshore development, all of which are growing in Southeast Asia (e.g., Todd *et al.* 2010; Schneider *et al.* 2015) and the Indo-Pacific (e.g., Edinger *et al.* 1998; Edinger *et al.* 2000). The range of *A. percula* is largely outside of areas that are experiencing the most rapid growth and industrialization, such as Indonesia and the Philippines. Throughout the range of *A. percula*, there are thousands of islands, many of which are uninhabited or have small, sparse human populations leading traditional lifestyles. These remote locations are unlikely to suffer from much exposure to increased sedimentation or nutrients. Williams *et al.* (2015) showed decreases in reef fish biomass with increasing human population densities and highest biomass at uninhabited islands. Most of Australia's reefs also lie far from large human populations. Even where there are population centers, notably along parts of the coast of Queensland, the reefs generally lie >30 km offshore (Burke *et al.* 2011). However, there is evidence that some of these remote and otherwise pristine areas in countries like Papua New Guinea and the Solomon Islands are targeted for intense or illegal logging and mining operations, which may be causing degradation of the nearshore environment, even in remote and uninhabited areas (e.g., Seed 1986; Kabutaulaka 2005). In a recent consensus statement, the Independent Science Panel of the Queensland Government declared that the decline of marine water quality associated with terrestrial runoff from the adjacent catchments is a major cause of the current poor state of many of the key marine ecosystems of the Great Barrier Reef (Brodie *et al.* 2013). The statement identifies agriculture as a diffuse source of excess nutrients, fine sediments and pesticides.

Organisms in coral reef ecosystems, including clownfish, are likely to experience continuing effects from anthropogenic sources of sedimentation and nutrient enrichment at some level as economies continue to grow. However, to date, efforts to examine the direct and indirect effects of nutrients and sedimentation to the orange clownfish throughout its range are lacking. Land-based sources of pollution on reefs act at primarily local and sometimes regional levels, with direct linkages to human population and land-use within adjacent areas. *Amphiprion percula* occur mostly in shallow reef areas and rarely migrate between anemone habitats as adults; these are traits that may make this species more susceptible to land-based sources of pollution in populated areas than other, more migratory or deeper-ranging reef fish. To account for the uncertainty associated with the magnitude of this threat, and consider the species' traits that may increase its susceptibility and exposure, we conservatively assign a low-to-medium likelihood that the threat is currently or will significantly contribute to extinction risk for *A. percula*. Spanning the low and medium categories indicates that the threat is likely to affect the species negatively and may have visible consequences at the species level either now and/or in the future, but we do not have enough confidence in the available information to determine the negative effect is of a sufficient magnitude to significantly increase extinction risk.

Factor B: Overutilization for Commercial, Recreational, Scientific or Educational Purposes

It is estimated that 1.5-2 million people worldwide keep marine aquaria, including 600,000 households in the United States (U.S.) alone (Wabnitz *et al.* 2003). Estimates place the value of the marine ornamental trade at approximately U.S. \$200-330 million per year (Wabnitz *et al.* 2003). The largest importers of coral reef fish, corals and invertebrates for display in aquaria are the U.S., followed by the European Union, Japan and China. The U.S. accounted for an average of 61% of global imports from 2000-2010 (Wood *et al.* 2012). A tremendous diversity and volume of species are involved in the marine aquarium trade (Rhyne *et al.* 2012). It is estimated that every year, approximately 14-30 million fish, 1.5 million live stony corals, and 9-10 million other invertebrates are removed from coral reef ecosystems across the world (Wood 2001a,b; Wabnitz *et al.* 2003; Tsounis *et al.* 2010) although Rhyne *et al.* (2012) assert that the volume of marine fish has been overestimated. These include the trade in at least 1,802 species of fish, more than 140 species of corals, and more than 500 species of non-coral invertebrates (Wabnitz *et al.* 2003; Rhyne *et al.* 2012). Clownfish, specifically *A. ocellaris* and *A. percula*, are among the top five most imported and exported species of marine aquarium fish in the aquarium trade (Wabnitz *et al.* 2003; Rhyne *et al.* 2012).

Collection in Papua New Guinea

Papua New Guinea did not have an aquarium fishery until 2007 when the National Fisheries Authority (NFA) commissioned a consulting company from the U.S. (EcoEZ Inc.) to do a resource assessment of marine species with the potential for the aquarium trade. Following the assessment, the project was funded for one year to develop a sustainable marine aquarium trade industry in Papua New Guinea. A total of 145 fishers in 8 communities were trained in proper collection techniques (Dandava-Oli *et al.* 2013). At the end of this first year, the project was extended for an additional two years, through 2010. Due to high operating costs, the project was shut down and no longer funded by the end of 2010. In 2011, detailed surveys of the Fishermen Island collection areas were conducted to assess fish, coral, and invertebrate abundance after the cessation of marine collection activities. Findings indicated that there were no significant differences between collection years and the 2011 assessment, indicating that collection areas were in good condition (Dandava-Oli *et al.* 2013). The NFA expressed concern at one point during the collection program because of many sea anemones spotted without their resident host clownfish, *A. percula*; however, more recent surveys found that few anemones were without clownfish, indicating the population had at least partially rebounded from previous collection pressure (Dandava-Oli *et al.* 2013). In early 2012, the NFA accepted a new proposal for a smaller operation called EcoAquariums. According to information on the EcoAquariums website, between November of 2011 and November of 2012, the company collected 15,000 fish, of which 30% were *A. percula* (4,970 individuals). However, the company shut down in 2013 due to the economic non-viability of operations, leaving Papua New Guinea without an active marine aquarium fishery for almost two years. The major hurdle for this industry in Papua New Guinea has consistently been high shipping and freight costs (Kinch 2008; Wabnitz *et al.* 2013). The Secretariat of the Pacific Community (SPC) conducted an assessment of marine aquarium activities in Papua New Guinea thus far and provided recommendations to the NFA regarding the future of the program including an economic viability assessment for aquarium trade activities, development of a solid business plan for future interested parties, and worked with the

NFA to finalize a management plan and accompanying regulations and licensing conditions should an aquarium fishery develop in the future (Dandava-Oli *et al.* 2013).

In summary, there is currently no export of marine ornamental species, including *A. percula*, from Papua New Guinea, where some of the highest species densities have been recorded. In 2014, a research effort began to evaluate the feasibility of establishing marine aquarium aquaculture in Papua New Guinea. Results as yet are few, but of note is that *A. percula* is the least affected by harvesting out of the species targeted thus far for this research (Thane Militz, pers. comm. 2015). This indicates that if *A. percula* collection in Papua New Guinea is resumed in the future, it will be informed by research, likely done sustainably, and may supply an aquaculture facility rather than support commercial export of wild caught individuals.

Collection in Vanuatu

Vanuatu is an archipelago that comprises approximately 80 islands. Collection of marine aquarium organisms has occurred in Vanuatu for over 20 years. While there is limited recent information on the industry in Vanuatu, two companies, Reef-Farm Vanuatu and Sustainable Reef Suppliers (SRS) were the main companies exporting fish, corals, live rock and invertebrates for the aquarium trade (SPC 2010). As of early 2015, only SRS is still active though its facilities and equipment, as well as the country's reefs, suffered major damage following the recent severe tropical cyclone, Pam, regarded as one of the worse natural disasters in the history of Vanuatu. According to both suppliers' websites (**Reef-Farm Vanuatu Ltd** and **Sustainable Reef Supplies Vanuatu**), *A. percula* is not listed as a collected species.

Collection in Solomon Islands

The Solomon Islands is an archipelago of nearly 1,000 islands, of which around 300 are inhabited. The export of marine species from the Solomon Islands began in the mid-1990s with an export company called Solomon Islands Marine Export (SIME). A few years later Aquarium Arts Solomon Islands (AASI) was founded, and the two companies exported the vast majority of the islands' live fish, corals and invertebrates destined for the aquarium trade (Kinch 2004a). Originally, 12 collection sites in the Western Province were identified, although as of 2004, only 2 of those sites remained active (Kinch 2004a). One of the collection areas in the Western Province was located at Madou in Vonavona Lagoon and was a local family-run operation. Collectors at this location specialized in anemonefish including *A. percula*. A report from 2004 shows 17,313 *A. percula* were purchased from fishers diving this collection area from 2002 through May of 2004, averaging 6,787 per year across the two complete years of data (Kinch 2004a). The second collection site, Rarumana, also listed *A. percula* as one of the primary target species. The same 2004 report indicates a total of 12,340 *A. percula* purchased from fishers collecting at this site from 2002 through May of 2004, with an average of 4,384 per year across the two years with complete data (Kinch 2004a). From the two locations combined, an average of 11,171 *A. percula* was purchased per year between 2002 and 2003. Seven communities in the Marau Sound area of Guadalcanal also collected fish as of 2004 (Kinch 2004b). However, there have been impediments to creating a profitable aquarium fishery in this area, including lack of capital and equipment, and the limited capacity and high cost of shipping and freight (Kinch 2004b).

While the numbers listed above are informative, they are more than 10 years old. Although very little published information is available to update the figures from 2004, consultation with experts has revealed some useful insights and the aquarium industry in the Solomon Islands has changed significantly over the last several years. Since 2004, SIME has gone out of business and AASI is the only company exporting live fish (and corals) in the Solomon Islands (Colette Wabnitz, pers. comm. 2015). AASI has undergone management changes, which together with periodic challenges linked to coral collection permitting at the government level have slowed operations since 2012. One of the key connections for transport between provinces (a small airport) is also no longer operational, meaning fewer fish are coming from the Western Province, although collection still occurs in Rarumana (Jeff Kinch, pers. comm. 2015). Additional collection still occurs around Guadalcanal in Marau and Ngella in the Central Province (Colette Wabnitz and Jeff Kinch, pers. comm. 2015). This information indicates that it is safe to assume collection of all species, including *A. percula*, still occurs, but has significantly decreased in the Solomon Islands over the last few years.

Collection in Australia/Great Barrier Reef

Aquarium fish have been collected from the Great Barrier Reef on a commercial basis since the beginning of the marine aquarium trade in the 1970s. The Queensland Marine Aquarium Fish Fishery (QMAFF) operates in an area from Cape York in the north and south to the New South Wales border; *A. percula*, however, only occurs in the northern portion of the Great Barrier Reef and only a small portion of its range occurs within the aquarium fishery area. No quantitative harvest or export information was available for review for *A. percula* in Australia, although a wealth of data are collected and reported from this fishery regularly and feed directly into sustainable management of the fishery. As an example, an assessment for the QMAFF was carried out in 2008 and identified *A. percula* as a species at low risk of overexploitation in the fishery (Roelofs 2008). An assessment in 2013 again declared *A. percula* as a species at low risk (Commonwealth of Australia 2014; State of Queensland 2014). As discussed above in section 3.3.1, Jones *et al.* (2008) found that collection has likely exacerbated the effects of bleaching and other disturbances on anemones and anemonefish in the Keppel Islands region, which is outside of the range of *A. percula*. In contrast, higher densities of anemones and anemonefish were recorded in the Far North Queensland region (within the range of *A. percula*) where collection also occurs, but fewer other disturbances have occurred.

Global Trade

Rhyne *et al.* (2012) reported a total of 400,000 individuals of the species complex *A. ocellaris/percula* were imported into the U.S. in 2005 (the species were combined due to common misidentification leading to the inability to separate them out in the import records). More recently, the author provided NMFS with updated estimates based on newer data from 2008-2011, which indicate the number of *A. percula* alone imported into the U.S. was less than 50,000 per year (Szczebak and Rhyne, unpublished). Notably, this estimate does not distinguish between wild-caught and captive-propagated individuals from foreign sources. The Philippines and Indonesia account for 80% of *A. percula* imports into the U.S. according to the new species-specific information from Szczebak and Rhyne (unpublished), and these countries are outside the geographic range of *A. percula*, indicating that 80% or more of the imported

individuals were likely propagated in captivity and not collected from the wild, or mis-identified. According to Tissot *et al.* (2010), the U.S. imports 50-70% of ornamental reef fish in the global trade. If we extrapolate the U.S. import estimate to infer global harvest for the aquarium trade, the number of globally traded *A. percula* in 2011 was likely closer to approximately 70,000-100,000 individuals, as many as 80% of which may be from aquaculture operations and not harvested from the wild, or mis-identified. Based on our conservative estimate of global population size of 13-18 million the collection of up to 100,000 *A. percula* (likely a vast over-estimate) throughout the species' range represents 0.0055% - 0.0076% of the population harvested annually.

Captive Propagation

Anemonefish were among the first coral reef fish raised in captivity throughout their entire life cycle and now represent one of the most well-known and well-developed captive breeding programs for marine fish (Dawes 2003). While no quantitative information was available to estimate the number of *A. percula* that are propagated in captivity, clownfish are widely described among the industry as an easily cultured aquarium species. In fact, an Internet search revealed numerous websites with instructions on how to breed clownfish in home aquaria. Oceans, Reefs, and Aquariums (ORA), the largest marine ornamental hatchery in North America, among others, cultures multiple species of clownfish, including *A. percula*, on a commercial scale; they note that clownfish were the first popular saltwater aquarium species to be cultured and have been bred for over 40 years (ORA 2015).

Another source of qualitative information to help inform our analysis is related to the preferences and demand of aquarium hobbyists. Many discussion boards, blogs, websites, and other information sources for home aquarists list numerous benefits of purchasing cultured clownfish instead of wild caught clownfish. Benefits of cultured fish include hearty individuals of known age that have not been exposed to parasites in the wild; fish that have not been exposed to the long arduous transport process that wild-caught fish undergo; fish that are acclimated to tank-style feeds; and fish that are typically less aggressive, less skittish, and are more visible and active in tanks (see the following hyperlinks: **Sea and Reef Aquaculture**; **Mad Hatters Reef**; **Saltwater Smarts**; and **The Reef Tank**; etc.). A survey of marine aquarium hobbyists in 2003 revealed that only 16% of respondents had no concern over whether they purchased wild vs. cultured organisms; the majority of respondents indicated a preference for purchasing captive bred specimens (Moe 2003). A more recent study reports that 76% of respondents to the same question indicated they would preferentially purchase cultured animals and an additional 21% said it would depend on the price difference (Murray and Watson 2014). Murray and Watson (2014) surveyed aquarium hobbyists and 85% of them indicated they have clownfish in their aquaria. They also did a gap analysis based on demand for certain species and whether or not they are good captive breeding candidates and assigned each species a "traffic light" color. Clownfish were assigned "green" because although there is high demand for the species, there are already a number of successful captive breeding programs in operation for these species.

Conclusion

In summary, *A. percula* are currently collected at varying levels in three out of the four countries in which the species occurs. Papua New Guinea had a fishery for this species, but does not

currently export for the aquarium trade. There is a small local aquarium industry but collection for this purpose is likely minimal (Colette Wabnitz, pers. comm. 2015). Research is ongoing to potentially re-open an aquaculture-based export industry out of Kavieng, Papua New Guinea. Collection from the wild appears relatively limited in Vanuatu, Australia, and the Solomon Islands, according to U.S. import information. While clownfish are targeted in these fisheries, they are not the most sought after species in most cases.

While almost 50,000 *A. percula* were imported into the U.S. alone in 2011, the majority of those were from countries outside the species' range indicating they were either mis-identified or from captive breeding facilities. Clownfish are easily propagated in captivity and are bred commercially by several of the largest reef fish suppliers in the U.S. and elsewhere. There appears to be a large and growing market for captive bred fish as consumers prefer fish of a known age that are already acclimated to a tank environment, and some wish to support reef conservation by reducing wild collection. Based on our conservative estimate of global wild population size of 13-18 million, the collection of up to 100,000 *A. percula* globally (likely a vast over-estimate extrapolated from the U.S. import estimate) throughout the species' range represents 0.0055% - 0.0076% of the population harvested annually. Based on the principles of fisheries management and population growth, we have determined that overutilization for commercial, recreational, scientific, or educational purposes poses a low risk of global extinction to *A. percula* now or in the foreseeable future.

Factor C: Disease or Predation

The available information on disease in *A. percula* indicates that the spread of some diseases is of concern in captive culture facilities (Ganeshamurthy *et al.* 2014; Siva *et al.* 2014); however, there is no information available indicating that disease may be a concern in wild populations. Captive cultured reef fish often experience rapid spreading of parasites, copepods, and other pathogens in captivity. *Amyloodinium ocellatum* is a parasitic dinoflagellate that causes "marine velvet disease" in aquacultured fish (Francis-Floyd and Floyd 2011). The parasitic copepod *Caligus longipedis* and the lymphocystis disease virus also are known to affect various fish species in aquaculture operations, including *A. percula* (Ganeshamurthy *et al.* 2014; Siva *et al.* 2014). Some pathogens that affect the species in captivity are likely to exist in and be introduced from the wild. However, cultured individuals are often stressed and stress increases an individual's susceptibility to pathogens. Close association in captivity also enables diseases to rapidly spread. Although diseases exist for captive *A. percula*, we could not find any records or reports of disease in wild *A. percula* populations. Because this is a well-studied species in at least parts of its range, we find this compelling evidence that disease does not currently pose a significant threat to the species. We therefore find that this threat is of very low importance to extinction risk for this species now and in the foreseeable future.

As for the threat of predation, *A. percula*, like many reef fish species, is most susceptible to natural predation in its egg, pelagic larvae, and settlement life stages. Shelter and protection from predators is one of the primary benefits conferred to post-settlement juvenile and adult *A. percula* by their symbiotic relationship with host anemones, as described above. We found no information to indicate elevated predation levels due to invasive species or other outside influences in any part of *A. percula*'s range is a cause for concern. Moreover, we did not find

any information to indicate that natural predation rates for the species are of a magnitude that would cause concern for their extinction risk now or in the foreseeable future.

As discussed below, there is some experimental evidence that indicates future levels of ocean acidification have the potential to negatively impact predator avoidance behavior for *A. percula*. However, it is unclear if or how those impacts may manifest themselves in the wild over the expected timeframes of increasing acidification, and there is evidence that trans-generational acclimation will play a role in allowing populations to adapt over time. While the future impacts of acidification are still unclear, we allow for the potential for impacts to predator avoidance behavior from ocean acidification by concluding that the likelihood of predation contributing to extinction risk for *A. percula* now or in the foreseeable future is low (instead of very low).

Factor D: Inadequacy of Existing Regulatory Mechanisms

Threats (factors) that specifically affect the species related to habitat destruction (e.g., sedimentation and nutrient enrichment), overutilization (e.g., collection for the aquarium trade), and other natural or manmade factors (e.g., effects from climate change) are discussed in their respective sections above. This section specifically addresses the lack of adequate regulatory mechanisms, or their enforcement, of those aforementioned threats.

Greenhouse Gas Regulations

While NMFS has acknowledged in several recent listing decisions that regulatory mechanisms are inadequate to address greenhouse gas emissions globally (see summaries at the following hyperlinks: **bumphead parrotfish management report** (NMFS 2012a); **coral management report** (NMFS 2012b); coral listing (79 FR 53851)), neither the habitat impacts nor the direct physiological impacts of global greenhouse gas emissions have risen to a level of concern for *A. percula* and its current or future extinction risk (see discussions above and below). As such, it is unlikely that the inadequacy of existing regulatory mechanisms for greenhouse gas emissions will contribute significantly to the extinction risk for this species now or in the foreseeable future.

Marine Aquarium Trade Regulations

Coral reef species are collected for the aquarium trade in at least 45 different countries around the world (see Wood 2001a,b; Smith *et al.* 2008; and Rhyne *et al.* 2012 cited in Thornhill 2012). Indonesia and the Philippines are the two largest exporters of coral reef organisms destined for the aquarium trade (Wood 2001b; Wabnitz *et al.* 2003; Rhyne *et al.* 2012). The marine aquarium trade industry as a whole is poorly regulated in several source countries, and imports and exports are generally poorly documented. There is a severe lack of data documenting the impacts of this global industry for the majority of traded coral reef species. Information, when it is available, is often haphazardly collected, out of date, or confounded by other problems (Thornhill 2012). However, of note is that recent efforts are underway to change this. Development and implementation of management plans are at different stages in a range of source countries throughout the South Pacific (e.g., Marshall Islands, Cook Islands, Papua New Guinea, Vanuatu, Tonga, Kiribati, and others). Also, efforts are being undertaken by the Secretariat of the Pacific Community (SPC) to have countries consistently record species and quantities in a standardized database. Progress is ongoing, though slow because of lack of capacity, staff shortages, and

challenges in the relationship between the private sector and government (Colette Wabnitz, pers. comm. 2015). Management and regulation of species collected for the marine aquarium trade are not sufficiently developed in most countries. Weak local and national governance capacity in major source countries, such as in Indonesia and the Philippines, combined with high international demand have resulted in limited and ineffective management (Tissot *et al.* 2010). On the other end of the industry, imports are poorly documented; Smith *et al.* (2008) found that in the U.S. from 2000-2005, only 3.8% of shipments of imported live fish were directly identified to the level of family, genus, or species. Often, labeling consisted only of general taxonomic designations such as “tropical marine species.”

Amphiprion percula does not occur in the waters of major source countries for wild reef fish exports (e.g., Indonesia and Philippines), with the exception of the northern coast of West Papua, which is on the western edge of its range. Within the countries where *A. percula* does occur, marine aquarium collection fisheries are relatively small-scale and generally have at least some government oversight in the form of licenses or permits. For example, in 2009, the Vanuatu Department of Fisheries worked with the SPC and the Pacific Islands Forum Fisheries Agency to develop a marine aquarium trade management plan. The aquarium fishery is now managed under this plan, which recognizes the importance of research in marine ornamental culturing for export. In the Solomon Islands, anemonefish were listed as prohibited exports under the Wildlife Protection and Management Act of 1998; however, this is currently not enforced and export of these species, including *A. percula*, continues (Kinch 2004a). In Australia, fisheries are limited entry, meaning a new entrant must purchase a fishing license, the total number of which is limited, before fishing can occur. As of 2013, there were 24 active licenses in the QMAFF out of 44 total licenses (Donnelly 2013). In Australia, Pro-Vision Reef, Inc. is an association of aquarium fish and coral collectors and their membership accounts for 91% of active licenses in the QMAFF. They have a close partnership with both the Great Barrier Reef Marine Park Authority and Fisheries Queensland, and together they developed the industry’s Stewardship Action Plan. The Action Plan places strong emphasis on minimizing ecological risks and maintaining healthy ecosystems upon which the industry depends. There are limits on the size of boats and number of divers used for collection activities, as well as several Special Management Areas in which no collection is allowed.

These regulated, small-scale fisheries, along with the prevalence of this species in commercial aquaculture operations, are factors contributing to our determination that overharvest for the marine aquarium trade has a very low likelihood of contributing to the extinction risk for this species. As such, it is unlikely that regulatory mechanisms related to marine aquarium collection and trade will contribute to the extinction risk for this species now or in the foreseeable future.

Sedimentation and Nutrient Enrichment Regulations

We evaluated the threat of land-based sources of impact to coral reefs (sedimentation and nutrient enrichment) on *A. percula* and determined that it has a low-to-medium likelihood of significantly contributing to the extinction risk for the species now and in the foreseeable future. Many regulatory mechanisms exist within *A. percula*’s range to address land-based sources of pollution with varying levels of efficacy and enforcement. Regulatory mechanisms for the four countries within *A. percula*’s range are described in detail in the NMFS coral management report (NMFS 2012b). Summaries are provided for each country below.

In Papua New Guinea, most legislation does not specifically refer to marine systems, which has generated some uncertainty as to how it should be applied to coral reefs. Also, the laws relevant to different sectors (e.g., fisheries, mining, environmental protection) are not fully integrated, which has led to confusion over which laws have priority, who is responsible for management, and the rights of the various interest groups.

Traditional management systems are still considerably important in the Solomon Islands, with all reefs being “owned” by particular groups with fishing rights under customary marine tenure. There are 11 Community Marine Conservation Areas that use customary sea tenure in locally adapted management strategies. The Fisheries Act of 1998 states that marine biodiversity, coastal and aquatic environments of the Solomon Islands shall be protected and managed in a sustainable manner and calls for the application of the precautionary approach to the conservation, management, and exploitation of fisheries resources in order to protect fisheries resources and preserve the marine environment (Aqorau 2005).

Customary tenure of reef resources is legally recognized in the Vanuatu constitution and via the Environmental Management and Conservation Act of 2002 (Republic of Vanuatu 2002). Each cultural group in Vanuatu has its own traditional approaches to management, which may include the establishment of marine protected areas, initiating taboo sites, or periodic closures. These traditional management schemes have been supplemented by various legislative initiatives, including the Foreshore Development Act, which regulates coastal development. The primary related responsibility for marine and coastal resource management in Vanuatu rests jointly with the Department of Fisheries within the Ministry of Agriculture, Quarantine, Forestry and Fisheries, and the Environment Unit within the Ministry of Lands and Natural Resources (Naviti and Aston 2000).

In Australia, *A. percula* occurs mostly, if not entirely, within the Great Barrier Reef Marine Park (GBRMP). In addition to the park, the Australian government has developed a National Cooperative Approach to Integrated Coastal Zone Management (Natural Resource Management Ministerial Council 2006). In response to recent reports showing declining water quality within the GBRMP, the State of Queensland recently developed and published a Reef Water Quality Protection Plan, outlining actions to secure the health and resilience of the Great Barrier Reef and adjacent catchments (State of Queensland 2013).

Overall, there is little information available on the enforcement or effectiveness of existing regulatory mechanisms addressing land-based sources of pollution throughout *A. percula*'s range. As such, it is difficult to determine the likelihood of the inadequacy of regulatory mechanisms contributing significantly to extinction risk for this species. To account for the uncertainty associated with this factor, we have determined the inadequacy of regulatory mechanisms addressing land-based sources of pollution has a low-to-medium likelihood of contributing to extinction risk for *A. percula*. Spanning the low and medium categories indicates that the threat is likely to affect the species negatively and may have visible consequences at the species level either now and/or in the future, but we do not have enough confidence in the available information to determine the negative effect is of a sufficient magnitude to significantly increase extinction risk.

Marine Protected Areas/Regulations

According to the IUCN's World Database of Protected Areas (WDPA), marine protected areas (MPAs) of all sizes exist throughout many areas of *A. percula*'s range in the Indo-Pacific. Though many MPAs exist, the overall effectiveness of these MPAs, let alone the conservation benefit that these MPAs provide specifically to *A. percula*, is unknown. However, experts generally agree that MPAs, if placed appropriately, are of sufficient size, and are enforced effectively, can enhance spawning stock biomass, allow for larval dispersal, maintain species diversity, preserve habitat, and sustain ecosystem function (e.g., Johnson *et al.* 1999; Russ and Alcala 1999; Bergen and Carr 2003; Shuman *et al.* 2005; McClanahan *et al.* 2006; Jupiter and Egli 2011). While there is some debate over whether many small MPAs or few large MPAs are more effective (e.g., Halpern 2003; Aswani and Hamilton 2004), there is widespread recognition that monitoring, evaluation, reporting and adaptive management are fundamental components of effective marine planning and management (e.g., Day 2008; Weeks and Jupiter 2013), regardless of size. There is also some empirical evidence that using indigenous ecological knowledge and existing customary management practices to design an MPA is showing signs of biological and social success for protecting coral reefs in Oceania (e.g., Aswani *et al.* 2007).

Though shortcomings of MPAs may exist (e.g., a great majority of MPAs worldwide fail to meet all of their management objectives (Jameson *et al.* 2002)), on average several biological measures (density, biomass, size of organisms, and diversity) are significantly higher inside reserves compared to outside (or after a reserve establishment versus before) (Halpern 2003). As suggested by Halpern (2003), nearly any marine habitat can benefit from the implementation of a reserve or MPA. As such, the following paragraphs describe the MPAs/regulations that occur throughout the four countries where *A. percula* resides. Empirical data on the overall effectiveness and enforcement of these specific MPAs/regulations, as well as the conservation benefit specifically to *A. percula*, is, however, lacking.

According to MPA Global, Papua New Guinea has 22 MPAs designated under national law (Wood 2007; NMFS 2012a). On a finer-scale, there are MPAs known as Locally Managed Marine Areas (LMMAs), which use indigenous ecological knowledge and customary management practices. LMMAs here and elsewhere are managed either by independent not-for-profit organizations, or by local village chiefs. For example, the Papua New Guinea Centre for Locally Managed Areas was formed in 2002. This non-profit organization is focused on helping communities to improve the practice of marine resource management within Papua New Guinea. Tools used within the MPA/LMMAs here and elsewhere include fishing gear restrictions, species-specific restrictions, and total no-take areas. The majority of MPAs/LMMAs in Papua New Guinea have been established around the edge of the Bismarck Sea (see Figure 3). Coral reefs in Papua New Guinea total 7,126 km² and the MPA/LMMA sites in the area total 4,550 km² (Coral Triangle Atlas 2012). Most recently, a network of nine LMMAs was established in Kimbe Bay, an area known for its high species diversity and high density of *A. percula*. These networks are linked through ocean currents, which promote resiliency for the coral reefs to withstand impacts from climate change (Green *et al.* 2009). Planes *et al.* (2009) assert that the MPA network in Kimbe Bay can function to sustain resident *A. percula* populations both by local replenishment and through larval dispersal from other reserves.

The Solomon Islands has nearly 1,000 islands, more than 2,802 km² of coral reefs, and 116 coral reef MPAs listed in the WDPA (NMFS 2012a) (see Figure 3). Due to the vastness of the archipelago, the Solomon Islands are still largely unaffected by human activities. Traditional management systems are of considerable importance and particular reefs are placed under restriction for periods of time (Spalding *et al.* 2001). The Solomon Islands LMMA Network was formed in 2003, with LMMAs in all 9 provinces in the area, totaling over 402 km² (Coral Triangle Atlas 2012) (see Figure 3). The Solomon Islands National Protected Areas Act also enables local communities to place their LMMAs under formal protection while restricting the activities of extractive industries (Coral Triangle Atlas 2012). Additionally, the eastern third of Rennell Island was declared a World Heritage Site in 1998, with boundaries extending seaward for 3 nautical miles and measuring 370 km² (Spalding *et al.* 2001). A number of MPAs that prohibit aquarium fishing also exist in the Vonavona Lagoon (Kinch 2004a).

Vanuatu consists of over 80 islands, 67 of which are inhabited by nearly 800 villages, with an average population of less than 200. There are 55 coral reef MPAs/LMMAs listed in the WDPA (NMFS 2012a) (see Figure 4). The Nguna-Pele Marine Protected Area Network manages the LMMAs. This network includes 16 indigenous communities engaged in conserving more than 3,000 hectares of marine and terrestrial resources. There is also the Vanuatu Village-based Resource Managed Areas Network, established in 2009. In addition to these LMMAs, customary tenure of reef resources is legally recognized in the Vanuatu constitution and via the Environmental Management and Conservation Act of 2002 (Republic of Vanuatu 2002). This includes initiating taboo sites, or periodic closures, within MPAs/LMMAs as a form of customary management used by individual communities (Caillaud *et al.* 2004). For example, Hickey and Johannes (2002) describe how in the early 1990s, the Vanuatu Fisheries Department promoted a voluntary, village-based *Trochus* sea snail management program. Only a few fishing villages were part of the original program, but after conservation success of the program, many villages decided to implement their own conservation measures to protect an array of marine species as well as implement fishing gear and use restrictions. By 2001, there were over 50 villages that had implemented marine resource management (MRM) activities. Dumas *et al.* (2010) investigated the effects of two very small (<0.05 km²) taboo areas located along the shallow fringing reef of Emau Island, Vanuatu. Surveys focused on heavily harvested species (namely *Trochus* giant clams and green snails) and results showed that under certain conditions, very small-scale reserves, such as those implemented by village-based conservation initiatives, can rapidly and efficiently enhance local reef invertebrate resources.

Lastly, in Australia, 75% of the total coral reef area is located within the 172 MPAs according to the WDPA (NMFS 2012a). In Queensland, the GBRMP Authority manages the comprehensive network of MPAs, which covers an expansive 345,400 km² of area under the authority of the Great Barrier Reef Marine Park Act of 1975 (see Figure 5). The GBRMP, designated as a World Heritage Site in 1981, has jurisdictional arrangements that overlap between the Australian Government and the Queensland Government. Strong cooperative partnerships have been built between these governments and commercial and recreational industries, research institutions and universities, all with complementary legislation and adaptive management of the GBRMP (UNESCO 2015). There are different levels of use allowed in various regions within the GBRMP, which can lead to differences in density of target species between open and closed areas (Jones *et al.* 2008). Although fisheries for many target marine species are highly regulated

in the GBRMP, no regulations exist specifically for clownfish. Additionally, the World Heritage status of the GBRMP has come under scrutiny recently by the United Nations Organization for Education, Science and Culture (UNESCO). The UNESCO World Heritage Committee working group “notes with concern” that the overall outlook for the reef is “poor,” and that climate change, poor water quality, and impacts from coastal development are major threats to its health and have been degrading key habitats, species, and ecosystem processes in the central and southern inshore areas (Dayton 2015).

In summary, a number of MPAs of varying degrees of size, management, and success exist throughout *A. percula*'s range. There is relatively little empirical data on the effectiveness of these particular MPAs; however, the general consensus is that these MPAs do provide some conservation benefits for marine species (e.g., Day 2002; McClanahan *et al.* 2006; McCook *et al.* 2010). In Vanuatu, Hickey and Johannes (2002) report success of LMMAs due to a variety of reasons, including enforcement. The authors report that there is an increasing use of state police to informally support decisions made by the village chiefs. Individuals who break these village taboos, including taboos relating to MRM activities, may be turned over to the police. More specifically regarding orange clownfish, findings suggest that the MPA network in Kimbe Bay, Papua New Guinea might function to sustain resident *A. percula* populations both by local replenishment and through larval dispersal from other reserves (e.g., Almany *et al.* 2007; Green *et al.* 2009; Planes *et al.* 2009; Berumen *et al.* 2012). Although we cannot determine the overall benefit to the species from the network of protected areas throughout its entire range, the mere existence of MPAs is likely to confer at least some conservation benefit and is unlikely to contribute to extinction risk for *A. percula* now or in the foreseeable future.

Factor E: Other Natural or Manmade Factors

The demographic risks assessed separately above in section 3.2 are considered in the analysis of Factor E. The assessment of those demographic risks found that they either have a low or very low likelihood of significantly affecting extinction risk for *A. percula*.

Physiological/Behavioral Impacts of Ocean Acidification

There is evidence that some species of reef fish may be affected by levels of ocean acidification predicted to occur before the end of this century. *Amphiprion percula*, along with several other pomacentrid species, has been the subject of several laboratory-based studies on this topic. The field of study is relatively new but here we summarize results that have been reported thus far.

Research has been focused on the effects of acidification on two aspects of physiology: (1) growth and development, and (2) sensory capabilities that affect behavior. In one study, increased acidification at levels expected to occur circa 2100 had no detectable effect on embryonic duration, egg survival, or size at hatching and, in fact, increased larval growth rate in *A. percula* (Munday *et al.* 2009a). Similarly, there was no effect on otolith size, shape, symmetry, or elemental chemistry when *A. percula* larvae were reared at CO₂ levels predicted by the year 2100 (Munday *et al.* 2011b). A different pomacentrid species, *Acanthochromus polyacanthus*, also showed no effect on juvenile growth, survival, skeletal elements or otolith size, shape, or symmetry when reared at high CO₂ levels comparable to those predicted as a result of climate change (Munday *et al.* 2011a).

When it comes to behavioral impairment, laboratory research has shown more consequential results regarding the potential impacts of future ocean acidification. An elevated CO₂ environment can affect auditory sensory capabilities for juvenile *A. percula*, even in the absence of effects on otolith growth. This indicates other possible mechanisms for this interference, such as deterioration of neural transmitters or compromised processing of sensory information (Simpson *et al.* 2011). Auditory sensory capabilities guide larval fish during settlement as nocturnal reef sounds promote settlement and daytime predator-rich noises discourage settlement (Simpson *et al.* 2011).

Increased CO₂ levels may affect olfactory cues used by larval clownfish to identify anemones and avoid predators. Larval clownfish use olfactory cues, such as odors from anemones, to locate suitable reef habitat for settlement (Munday *et al.* 2009b). Larval *A. percula* reared at CO₂ levels comparable to those predicted by the end of this century showed no observable response to olfactory cues of different habitat types, whereas those reared in the control environment showed a strong preference for anemone olfactory cues over other habitat olfactory cues (Munday *et al.* 2009b). Newly hatched *A. percula* larvae also innately detect predators using olfactory cues, and they retain this ability through settlement (Dixson *et al.* 2010). When tested for behavioral responses to olfactory cues from predators, *A. percula* larvae raised in both the control environment (390 ppm CO₂) and the lower of the two intermediate environments tested (550 ppm CO₂) showed strong avoidance of predator cues. However, larvae reared at 700 ppm CO₂ showed variation in their responses, with half showing avoidance of predatory cues and the other half showing preference for predator cues (Munday *et al.* 2010). In this same study, larvae reared at 850 ppm showed strong preference for predator cues, indicating that 700 ppm CO₂ may be a threshold at which adaptation is possible or natural selection will take effect because of the mixed responses to olfactory cues (Munday *et al.* 2010). Additionally, Dixson *et al.* (2010) report that CO₂ exposure at the egg stage does not appear to affect olfactory sensory capabilities of hatched larvae, but these capabilities are affected when settlement stage larvae are exposed to elevated CO₂.

The results discussed above indicate that ocean acidification associated with climate change has the potential to affect behavioral responses of *A. percula* to certain cues during critical life stages. However, if or how these effects will manifest at the population level requires an understanding of additional factors. All of the aforementioned authors acknowledge that the potential for acclimation or adaptation was not factored into their studies because it is generally unknown or hard to predict. Additionally, the authors did not measure possible effects of the synergy between increases in temperature and acidification. Murray *et al.* (2014) assert that there is mounting evidence of an important but understudied link between parent and offspring generations, known as parental conditioning or trans-generational plasticity (TGP), which may comprise a short-term adaptation mechanism to environmental acidification. This type of plasticity describes the ability of the parental environment prior to fertilization to influence offspring reaction norms without requiring changes in DNA sequence (Salinas and Munch 2012). Trans-generational plasticity in CO₂ resistance as a potential adaptation for coping with highly variable aquatic CO₂ environments may be common (Salinas and Munch 2012; Dupont *et al.* 2013). One recent study found that the effects associated with rearing larval clownfish (*A. melanopus*) at high CO₂ levels, including smaller length and mass of fish and higher resting metabolic rates, were absent or reversed when both parents and offspring were reared in elevated

CO₂ levels (Miller *et al.* 2012). These results show that non-genetic parental effects can have a significant influence on the performance of juveniles exposed to high CO₂ levels with the potential to fully compensate for the observed effects caused by acute (within generation) exposure to increased CO₂ levels (Miller *et al.* 2012). Murray *et al.* (2014) report the first evidence of TGP in a wild population of Atlantic silverside (*Menidia menidia*). Their results showed that within one breeding season, seasonal fluctuations in CO₂ levels in the environment led to different tolerances of increased CO₂ conditions for juveniles; those born early in the season when environmental CO₂ levels are low had reduced survival at high CO₂ treatment levels, but offspring from parents collected later in the season, when environmental CO₂ levels were naturally elevated, had equal survival at all CO₂ treatment levels (Murray *et al.* 2014).

In addition to the potential for acclimation and TGP, it is difficult to interpret the results of laboratory studies of acute exposure in terms of what is likely to happen in the foreseeable future in the wild or to predict potential population level effects for a species. The acute nature of the exposure and acclimation in the studies above is noteworthy because most species will not experience changes in acidification so acutely in their natural habitats. Rather, they are likely to experience a gradual increase in average CO₂ levels over several generations, and therefore parental effects could be highly effective in moderating impacts. Moreover, there is ample evidence that coral reef ecosystems naturally experience wide fluctuations in pH on a diurnal basis (Gagliano *et al.* 2010; Gray *et al.* 2012; Price *et al.* 2012). Price *et al.* (2012) found that reefs experienced substantial diel fluctuations in temperature and pH similar to the magnitudes of warming and acidification expected over the next century. The pH of ocean surface water has decreased from an average of 8.2 to 8.1 (decrease of 0.1) since the beginning of the industrial era (IPCC 2013). The pH of reef water can vary substantially throughout the day, sometimes reaching levels below 8.0 in the early morning due to accumulated respiration of reef organisms in shallow water overnight (Ohde and van Woesik 1999; Kuffner *et al.* 2007). Primary producers, including zooxanthellae in corals, uptake dissolved CO₂ and produce O₂ and organic matter during the day, while at night respiration invokes net CO₂ release into the surrounding seawater. In fact, Ohde and van Woesik (1999) found one site that fluctuated between pH 8.7 and 7.9 over the course a single day.

We conclude that the threat of physiological impacts from ocean acidification has a low likelihood of having a significant effect on the species' risk of extinction now or in the foreseeable future. Studies clearly show that in a controlled setting, an increased CO₂ environment can impair larval sensory capabilities that are required to make important decisions during critical life stages. However, a disconnect exists between these experimental results and what can be expected to occur in the wild over time, or even what is currently experienced on a daily basis on reefs. Even though projections for future levels of acidification go out to the year 2100, we do not consider the effects of this potential threat to be foreseeable over that timeframe due to the variable and uncertain nature of effects shown in laboratory studies versus what the species is likely to experience in nature over several generations. The species has demonstrated susceptibility to ocean acidification in laboratory studies. However, there is evidence from a congener that susceptibility decreased or disappeared when tested over several generations. There is uncertainty associated with *A. percula*'s likely level of exposure to this threat in the foreseeable future given the uncertainty in future ocean acidification rates and the heterogeneity of the species' habitat and current environmental conditions across its range. The best available information does not indicate that ocean acidification is currently creating an extinction risk for

the petitioned species in the wild through impacts to fitness of a significant magnitude. Further, we do not have sufficient information to suggest future ocean acidification will significantly affect the extinction risk for *A. percula* in the foreseeable future.

Physiological Impacts of Ocean Warming

The best available information does not indicate that ocean warming is currently creating an extinction risk for *A. percula* in the wild through impacts to fitness of a significant magnitude. While it has yet to be studied specifically for *A. percula*, researchers have begun to explore the potential impact of increasing temperature on the physiology of other pomacentrid species. *Dascyllus reticulatus* adults exposed to a high temperature (32° C) environment in a laboratory setting displayed significantly reduced swimming and metabolic performance (Johansen and Jones 2011). Other results include reduced breeding success of *Acanthochromis polyacanthus* (Donelson *et al.* 2010) and increased mortality rates among juvenile *Dascyllus aruanus* (Pini *et al.* 2011) in response to increased water temperatures that may be experienced later this century. Multiple references on the subject state that the effects of temperature changes appear to be species-specific (Nilsson *et al.* 2009; Lo-Yat *et al.* 2010; Johansen and Jones 2011); therefore, these results are not easily applied to *A. percula*. With regard to ocean warming impacts to respiratory and metabolic processes, Nilsson *et al.* (2009) and Johansen and Jones (2011) compared results of exposure to increased temperatures across multiple families or genera and species of reef fish. Both studies reported negative responses but the magnitude of the effect varied greatly among closely related species and genera. As such, it is difficult to draw analogies to unstudied species like *A. percula*. As with acidification, Price *et al.* (2012) found that reefs experienced substantial diel fluctuations in temperature similar to the magnitude of warming expected over the next century. In addition, TGP in temperature-dependent growth was recently documented for two fish species, where offspring performed better at higher temperatures if the parents had experienced these temperatures as well (Donelson *et al.* 2011; Salinas and Munch 2012). There is epistemic uncertainty associated with the threat of future ocean warming to *A. percula*. Susceptibility of reef fish that have been studied varies widely and the role TGP may play in acclimation over time is unknown. In addition, we cannot predict the exposure of the species to this threat over time given the uncertainty in future temperature predictions and the heterogeneity of the species' habitat and current environmental conditions across its range. Further, we do not have sufficient information to suggest future ocean warming will significantly affect the extinction risk for *A. percula* in the foreseeable future.

Summary of Threats

Table 2 below summarizes our assessment of the likelihood that each threat contributes or will contribute to extinction risk for *A. percula*. For the threats to which we assigned a low likelihood, we determined based on the best available information that the factor was unlikely to contribute significantly to extinction risk for *A. percula*. For some threats, we considered this a conservative assignment of likelihood to account for some uncertainty associated with either the magnitude of the threat or the exposure of *A. percula* to the threat. We assigned a very low likelihood to threats where more information was available that provided more certainty about their likely magnitude and therefore their impact on extinction risk. We assigned a low-to-medium likelihood to sedimentation and nutrient enrichment and the inadequacy of regulatory mechanisms associated with this threat because this is a present and ongoing threat likely to

worsen in the future. In addition, *A. percula* has traits that render it more susceptible to land-based sources of pollution, although its overall exposure is uncertain.

Conservation efforts

Higher rates of exploitation for wild orange clownfish have occurred in the past but recent data indicates that exploitation rates may be declining. Approximately 400,000 individuals of the species complex *A. ocellaris/percula* were imported into the U.S. (the largest importer of the species) in 2005 (Rhyne *et al.* 2012). This includes both wild-caught and captive-propagated individuals. More recent data specific to *A. percula* estimates that fewer than 50,000 individuals per year were imported into the U.S. from 2008-2011 (Szczebak and Rhyne, unpublished). When extrapolating out to infer quantity in the global aquarium trade, the number of globally traded *A. percula* during this time period was likely anywhere from 70,000-100,000 individuals, with as many as 80% from countries outside the species' range, thus likely either from captive propagation and not harvested from the wild or are mis-identified.

The shift from wild-caught exports of *A. percula* to captive-propagated individuals is supported by the available information about the marine aquarium industry throughout the species' range and corroborated by survey data from hobbyists. As described previously in section 3.3.2, collection efforts in Papua New Guinea, the Solomon Islands, and Vanuatu have declined over the past decade for a variety of reasons.

As of 2014, Papua New Guinea is actively supporting a research project with the ultimate goal of re-entering the marine aquarium industry. Research topics include both sustainable catch limits and aquaculture production of popular species like *A. percula* (Militz 2015). Specific research objectives include answering the following questions: (1) How does wild harvest of key anemonefish of value to the ornamental trade (including *A. percula*) influence abundance and population composition; (2) how can we establish the reproductive biology and ontogeny of key anemonefish under culture conditions and with different diets; (3) would restocking cultured anemonefish onto reefs impacted by commercial collection ameliorate the impacts of capture; (4) would the chemical agent bisazir, used to sterilize temperate fish, be effective in sterilizing marine ornamental fish; and (5) do cultured fish experience less stress from ocean to aquarium than wild caught individuals (Thane Militz, pers. comm. 2015). As of the writing of this status review, data collection for question one is complete but the results are not yet published. The researcher provided some general summary information. He noted that the primary problem with clownfish collection in general is not simply the removal of the fish but rather competing low-value, less targeted species (*A. clarkii*, *A. melanopus*, *A. perideraion*) taking over anemones where fish have been removed and prohibiting recruitment of the original species. That being said, he noted that *A. percula* was the least affected by harvesting in the first part of the study in Papua New Guinea because it is almost exclusively hosted by *S. gigantea* anemones, so other species did not take over once *A. percula* was removed. Additionally, while *H. magnifica* anemones are utilized by both *A. percula* and *A. perideraion*, the two fish species seem to have quite different habitat preferences, as supported by Elliott and Mariscal (2001). As opposed to the general observation of replacement by other species for most collected clownfish, results of this research saw little replacement by *A. perideraion* when exploiting *A. percula* (Thane Militz, pers. comm. 2015).

A collaborative research project was started in 2008 between the Vanuatu Fisheries Department, SPC, and the Japanese International Cooperation Agency and focused on the production of clownfish species in captivity (Kinch and Teitelbaum 2008). This project was, however, short-lived and Vanuatu currently does not export captive-bred specimens (Colette Wabnitz, pers. com. 2015).

In Australia, the aquarium fishery undergoes periodic ecological risk assessments to ensure that harvested resources are managed sustainably. In the 2008 assessment report, *A. percula* was considered a species that is at a low risk of overexploitation in the fishery (Roelofs 2008). The species was again assessed as low risk in a 2013 assessment report (Commonwealth of Australia 2014; State of Queensland 2014).

While export of *A. percula* still occurs throughout the species' range, we can assume that overall harvest levels have decreased over recent years. Quantitative information on captive-propagated *A. percula* is lacking; however, anecdotal evidence indicates that clownfish, including *A. percula*, are easily cultured for commercial trade. Oceans Reefs and Aquariums, a commercial aquaculture company, notes that "most reliable fish dealers will sell only tank-raised clownfish, knowing their customers will benefit" (ORA 2015) because wild-caught clownfish historically have higher than average mortality after transport. Additionally, captive-bred individuals are typically much less expensive than wild caught (Live Aquaria 2015). As such, captive broodstock of *A. percula* from countries such as Indonesia as well as within the U.S. are being used not only to meet the commercial demands for the species, including providing hardier and less expensive individuals, but also serve as a conservation tool to alleviate or reduce the demand from wild populations throughout the species' range. Anecdotal Internet discussion board content and formal survey results both indicate that a significant proportion of aquarium hobbyists and owners preferentially purchase captive-propagated fish because of their higher quality and the conservation benefits.

CONCLUSIONS

In assessing four demographic risks for *A. percula* -- abundance, growth rate/productivity, spatial structure/connectivity, and diversity -- we determined that the likelihood of three of these risks individually contributing in a significant way to the extinction risk for the species is low, and unknown for the fourth risk. We acknowledge that uncertainties exist regarding how these demographic risks may affect the species on an individual and population level. However, the species' estimated wild abundance of 13-18 million individuals is at a level sufficient to withstand demographic stochasticity. Moreover, productivity appears to be at or above replacement levels, rates of dispersal and recruitment at the local scale appear sufficient to sustain meta-population structure (although global genetic structure is unknown), and species diversity may allow for trans-generational adaptation to environmental variability. As such, even with acknowledgement of uncertainties, we have determined that these demographic risks have a low or unknown likelihood of contributing in a significant way to the extinction risk of *A. percula*.

We also assessed current and predicted threats to the species and determined that the likelihood of these individual threats contributing to the extinction risk of the species throughout its range vary between very low and low-to-medium. We again acknowledge uncertainties in predicting the breadth of the threats and the extent of the species' response, but we can assume these threats are reasonably certain to occur at some magnitude. For some threats, such as anemone bleaching, evidence indicates these events will become more severe and more frequent over the next few decades (van Hooijdonk *et al.* 2013). However, anemone susceptibility and response is variable, and *A. percula* is known to associate with five anemone hosts, indicating that the species may be resilient to this threat. Additionally, the species may exhibit resiliency and adaptation to threats such as ocean acidification and ocean warming via trans-generational plasticity. While it is unknown how much adaptation the species will undergo, we anticipate such threats to occur over space and time rather than acutely.

Of the 12 identified current and predicted threats, our greatest concern relates to the species' susceptibility and exposure to sedimentation and nutrients, as well as the inadequacy of regulatory mechanisms to address this threat, especially since juveniles and adults occur in shallow water and are non-migratory once they have settled into a host anemone. Therefore, we conservatively assigned a low-to-medium likelihood that both this threat and the inadequate regulatory mechanisms to address this threat may significantly contribute to the extinction risk for *A. percula*.

We have determined that the overall extinction risk to *A. percula* is low, both now and in the foreseeable future. We recognize that some of the demographic risks and threats to the species may work in combination to produce cumulative effects. For example, increased ocean acidification may affect the olfactory and auditory sensory capabilities of the species and potentially affect predation rates; ocean warming may affect the aerobic capacity of the species or the rates of disease; and harvest of sea anemones may eliminate important habitat for the species and potentially increase the likelihood of predation; and therefore, interactions within and among these threats may affect individuals of the species. However, despite our acknowledged uncertainties, even these synergistic effects that can be reasonably expected to occur from multiple threats and/or demographic risks are not anticipated to rise to the level of significantly affecting the extinction risk for this species. Individuals may be affected but we do not anticipate the species to respond to these threats in a way that may cause measurable impacts at the population level. The range of the species across heterogeneous habitats, the conservatively estimated abundance of 13-18 million individuals, the temporal variation in threats, coupled with the resiliency and trans-generational adaptive capabilities of the species to potential impacts contribute to a low overall vulnerability of the species.

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Table 1.-- Summary of demographic risk factors for *A. percula* and relative strength of the evidence indicating these factors are posing an extinction risk for the species. Characterizations of the relative likelihood (very low, low, medium, high, or unknown) that a particular factor is contributing in a significant way to the extinction risk of the species are explained further in Section 3.2.4.

Demographic Risk	Likelihood
Abundance	Low*
Growth rate / productivity	Low*
Spatial structure and connectivity	Unknown
Diversity	Low*

*Low means it is unlikely that the particular threat contributes or will contribute significantly to extinction risk for the species.

Table 2.--Summary of threats for *A. percula* and relative strength of the evidence indicating these factors are posing an extinction risk for the species. Characterizations of the relative likelihood (very low, low, medium, high, unknown) that a particular factor is contributing in a significant way to the extinction risk of the species are explained further in Section 3.3.6..

	Threat	Likelihood
Habitat	Anemone Bleaching	Low**
	Anemone Collection	Low**
	Sedimentation and Nutrient Enrichment	Low-to-Medium±
Over-utilization	Collection for the Aquarium Trade	Low**
Disease or Predation	Disease	Very Low*
	Predation	Low**
Inadequate Regulations	Greenhouse Gas Emissions Regulations	Low**
	Marine Aquarium Trade Regulations	Low**
	Sedimentation and Nutrient Enrichment Regulations	Low-to-Medium±
	Marine Protected Area Regulations	Low**
Other	Physiological/Behavioral Impacts of Ocean Acidification	Low**
	Physiological Impacts of Ocean Warming	Low**

*Very Low means it is very unlikely that the particular threat contributes or will contribute significantly to the extinction risk for the species.

**Low means it is unlikely that the particular threat contributes or will contribute significantly to the extinction risk for the species.

±Low-to-Medium means it is somewhere between unlikely and likely (i.e., possible but not necessarily probable) that the particular threat contributes or will contribute significantly to the extinction risk for the species.



Figure 1.--Photograph of *A. percula*. © www.aquaticcreationsgroup.com.

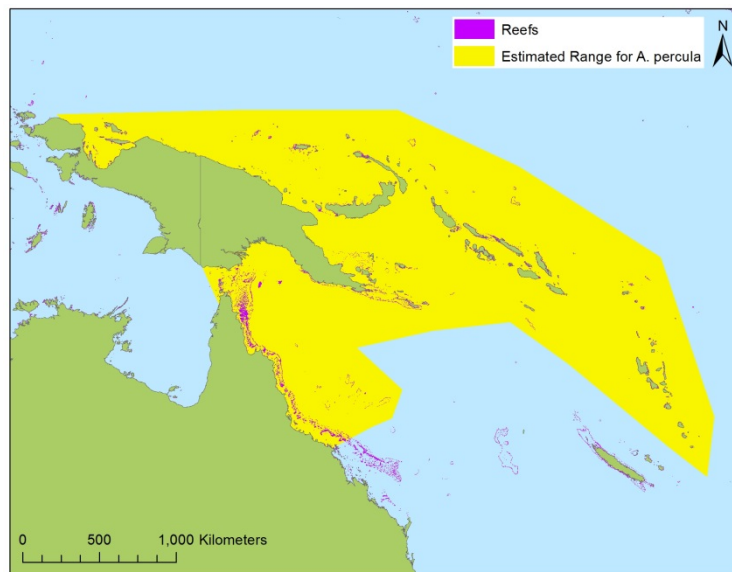


Figure 2.--Range map for *A. percula* based on Fishbase.org, Timm *et al.* 2008, and De Brauwer 2014, also showing coral reef areas from Burke *et al.* 2011.

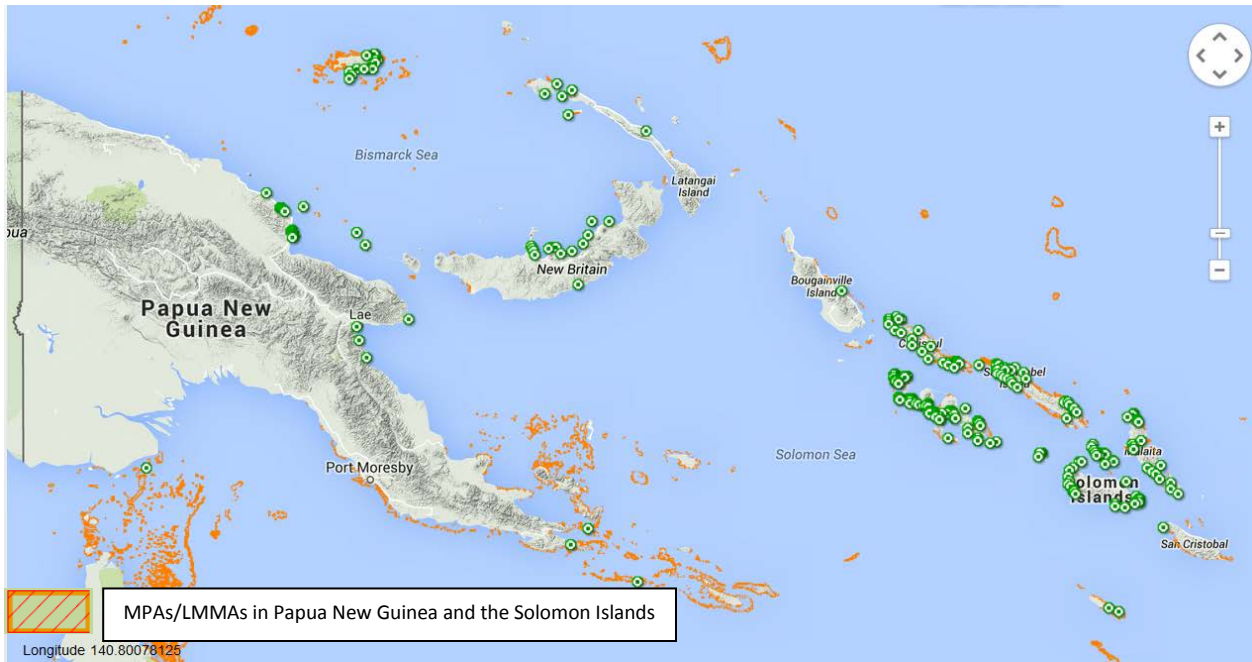


Figure 3.--General depiction of MPAs/LMMAs in Papua New Guinea and the Solomon Islands. Note that not all protected areas for both countries are shown. Green circles represent MPAs/LMMAs and orange areas represent coral reef areas. Map courtesy of the Coral Triangle Atlas interactive map available at <http://ctatlas.reefbase.org/atlas/default.aspx?layers=8,68>.

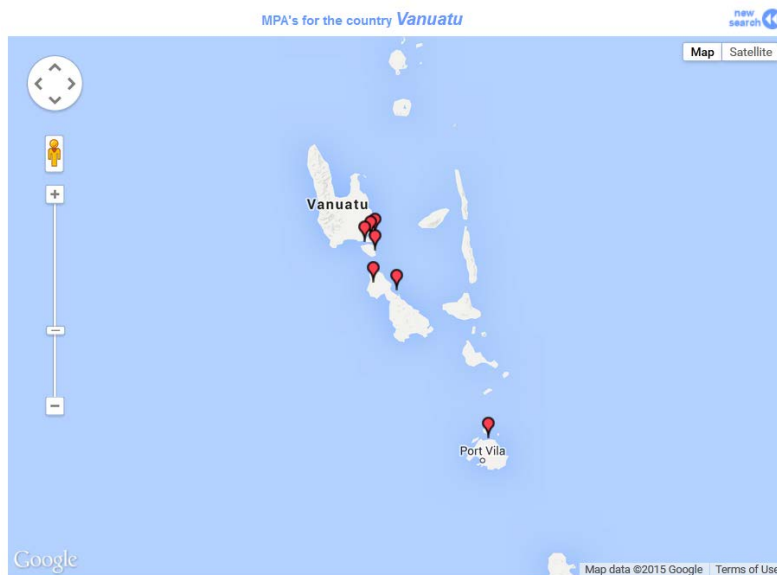


Figure 4.--General depiction of MPAs/LMMAs in Vanuatu. Note that not all MPAs/LMMAs are shown. Map courtesy of MPA Global (Wood 2007).

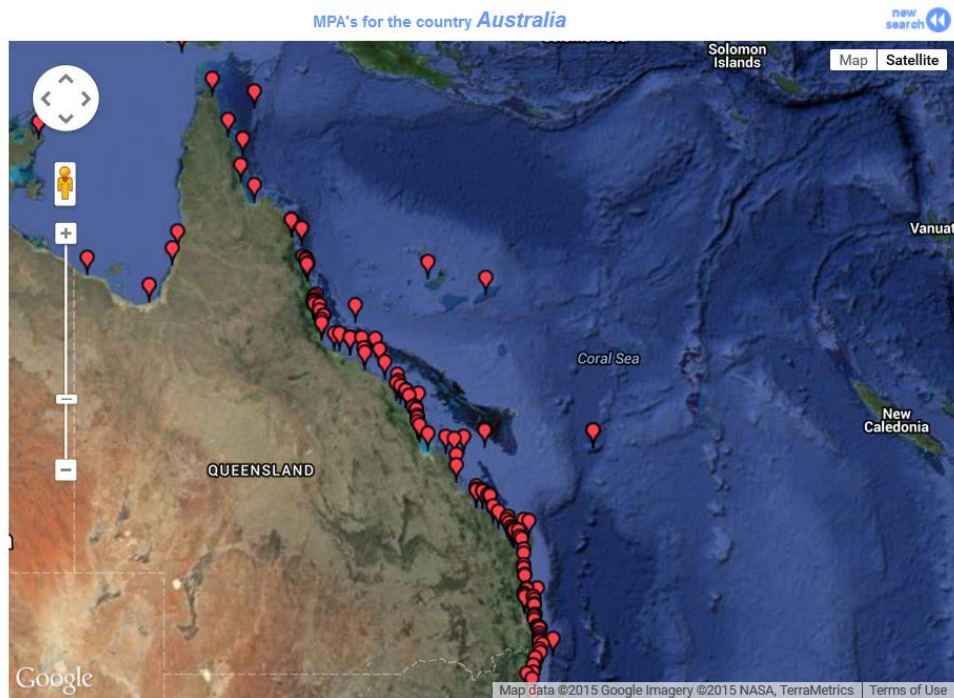


Figure 5.--General depiction of MPAs in Queensland, Australia. Note that not all MPAs are shown. Map courtesy of MPA Global (Wood 2007).

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