

Sea Anemones and Anemonefishes: A Symbiotic Relationship

by

Alicia Opala

(Biol 515 at SDSU, Fall 2003)

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INTRODUCTION

Symbiotic relationships are of great ecological importance, and some are vital to the well-being of all living things, such as, the symbiotic organisms living within the bodies of herbivores (Perry 1983). Many of these symbiotic relationships are not very well known or obvious, such as the relationship between butterflies and ants (Perry 1983). Other relationships are well known and easily seen, such as between Cow birds and cattle (Perry 1983). According to Roughgarden (1975), one of the most common and well known associations that has prompted much research is between sea anemones and the anemonefishes. Sea anemones (order Actiniaria), host anemonefishes (genus *Amphiprion*) providing them with shelter and a variety of other benefits. In turn, the anemonefishes provide the anemones with certain advantages (Roughgarden 1975). This paper discusses this relationship between sea anemones and anemonefishes, focusing mainly on the mechanism of how this relationship takes place and on the advantages gained by both animals.

SEA ANEMONES, ANEMONEFISHES, AND SPECIES INTERACTIONS

Sea anemones (order Actiniaria) are large solitary polyps found exclusively in marine environments. The sea anemone body consists of a cylinder shaped muscular column that expands at one end into a flat oral disk surrounded by tentacles with a mouth in the center (Pearse et al. 1987). These tentacles are studded with nematocysts which are used in capturing prey, transferring food to the mouth and for defense (Pearse et al. 1987). The opposite end of the body is a mucous coated basal disk which the anemone uses to attach tightly to the substratum (Pearse et al. 1987). There are a variety of characteristics for identifying anemone genera and species in the field, including tentacle number, form, and arrangement; color pattern, presence or absence of verrucae, habitat, and fish symbionts (Fautin 1991). Sea anemones are found worldwide in a variety of habitats, however, because of the distribution of anemonefishes, only the anemones found in tropical waters host anemonefishes (Elliott 1995, Astakhov 2002).

According to Allen and Fautin (1992), there are ten tropical species of anemones that host fish symbionts, and compared to the anemonefishes that all belong to the same genus, the species of anemones are not all closely related. These anemones belong to five genera from three families: Actiniidae which includes the species *Entacmaea quadricolor* and *Macrodactyla doreensis*, Stichodactylidae which includes *Stichodactyla gigantea*, *S. haddoni*, *S. mertensii*, *Heteractis aurora*, *H. crispa*, *H. magnifica*, and *H. malu*, and the family Thalassianthidae which includes *Cryptodendrum adhaesivum* (Astakhov 2002). The anemones that host anemonefishes are usually located in coral reefs or in close proximity to them, and different species of anemones are found in certain zones of the reefs, for example different depths or substrata (Astakhov 2002, Richardson 1999).

Anemonefishes belong to the genus *Amphiprion*, which is in the family Pomacentridae (Mariscal 1970). This genus is comprised of twenty-six species to date (Elliot 1995). *Amphiprion* are found in shallow waters throughout the Indo-West Pacific faunal region with the exception of the Hawaiian Islands, Johnston Island, Line Islands, Marquesas Islands, Pitcairn, Rapa, and Easter Island. The greatest concentration of species is located in the Indo-Australian Archipelago-Philippines region (Allen 1972).

In addition to their similar morphology and location, the relationship with sea anemones is probably the most well known feature that unifies members of this genus (Allen 1972). Members of the genus *Amphiprion* are dependent on the anemones for shelter and protection and are never found in nature without an anemone host (Fautin 1991). The interaction between these two organisms takes place through a natural and still not clearly defined mechanism that will be discussed in the following sections.

THE SYMBIOTIC RELATIONSHIP

The relationship between sea anemones and anemonefishes is a type of symbiosis, which basically means “living together”. Symbiotic relationships are always inter-specific, meaning that they occur only between different species (Perry 1983). Symbiotic relationships are divided into three main types: parasitism, commensalism, and mutualism (Deloach 1999). In a parasitic relationship, one species benefits and the other is either harmed or potentially harmed in the process. A commensalistic interaction is one in which one species benefits and the other is neither helped nor harmed. In contrast to the other two types of symbiosis, a mutualistic relationship is one in which both species benefit; this is the type of symbiosis that takes place between sea anemones and anemonefishes (Deloach 1999, Boucher 1982).

The degree of specificity in a symbiotic relationship depends on the number of host species that a symbiotic organism will live and interact with in nature (Miyagawa 1989). Most members of *Amphiprion* live with only a few host anemone species, but one complex, *A. clarkii*, has been found living with up to ten different host anemone species (Elliott 1998, Mitchell 2003).

Evolution of the Anemone-Anemonefish Symbiosis

It has been suggested by past research that the relationship between sea anemones and anemonefishes started with the ancestral anemonefishes being host generalists that were able to exploit many different sea anemone species (Elliott 1998). These species would have been good swimmers and less dependent on their relationship with sea anemones for protection (Allen 1972, Elliott 1998). Once this ancestor became adapted to living with the host anemones, evolution allowed anemonefishes to radiate into a variety of specific niches by “becoming more specialized for living with particular host species” of anemones (Elliott 1998). These more specialized anemonefishes showed an evolutionary change in morphology such as having slender body forms compared to the ancestral forms, being poor swimmers, and being highly dependent on the anemone hosts for protection (Allen 1972, Elliott 1998). According to Elliott (1998) these anemonefishes live with only one or two specific hosts.

However, in recent studies this hypothesis has proved to be incorrect. According to Elliott (1998), when recent molecular data was analyzed, it was found that the ancestral anemonefishes,

members of the genus *Premnas*, were host specialists rather than generalists as previously suggested. Therefore, host generalization is a derived trait that evolved in the clade leading to *A. clarkii*, while other clades evolved towards more specialization for living with certain other anemone host species (Elliott 1998, Carter 2000). For example, *A. clarkii* is attracted to most anemone species and can live symbiotically with them; however, *A. ocellaris* is usually only found living with the anemones *Heteractis magnifica* or *Stichodactyla gigantea* (Elliott 1998).

The morphology of the fins of these anemonefishes reflects whether they live with many or only a few species of host anemones (Allen 1972, Elliott 1998). Anemonefishes that are host specialists exhibit rounded caudal fins that are not as effective for fast swimming as are fins that are truncate or emarginated, which are exhibited by *A. clarkii*, a host generalist (Elliott 1998). It is thought that the ancestral anemonefish received high levels of protection from its anemone and therefore allowed for adaptation of the body and caudal fin for effective feeding instead of escaping predators (Elliott 1998).

Mechanisms Controlling Host Specificity

It is believed that host-specificity patterns are the result of the recognition of the host species by larval anemonefishes (Arvedlund 1999, Murata 1986). There are two theories as to how this recognition takes place. The first theory is that a visual cue such as color of the host anemone attracts larval anemonefishes to a specific host (Arvedlund 1999). The second theory is that chemical cues attract larval anemonefishes to a host anemone. Experiments comparing host recognition by visual cues versus chemical cues tested these theories (Arvedlund 1999, Murata 1986). These experiments showed that larval anemonefishes use chemical cues to recognize specific host anemones. It has been suggested that embryos developing in egg cases beside the anemone in which the parents live may imprint to olfactory cues released from the host anemones, thus allowing the newly hatched larval fish to recognize the anemone species (Miyagawa 1989). Miyagawa (1989) conducted an experiment which showed that different species of anemonefishes can recognize the same species of anemone by different chemical cues. This finding suggests that there are a variety of chemicals differing in structural type, activity level, and function that are involved in maintaining the species-specific relationship between these two animals. Some of the chemicals that Miyagawa (1989) was able to isolate from different anemones are: amphikuemin, tyramine 4, and tryptamine 5.

If the larval anemonefishes imprint on their host anemones via chemical cues, then this may have an influence on the spawning sites picked by the adult anemonefishes (Arvedlund 2000). According to Miyagawa (1988), Mitchell (2003), and Arvedlund (2000), anemonefish parents always laid their eggs within close proximity to the host anemone's column or pedal disk, with the tentacles hiding and touching the eggs. Most of the spawning sites are found towards the sea surface, uphill above the anemone. Arvedlund (2000) gives two possible explanations for this preference in spawning site. The first is that this choice of sites may encourage host imprinting. This position of the eggs in relation to the anemones may allow for the greatest amount of chemical released from the anemone to carry over the eggs, allowing the embryos to imprint on the host that released the chemicals. An alternative theory is that the anemones tentacles provide protection to the eggs. Because the eggs are already protected from predators by the very territorial parents, however, protection by tentacles may have less influence on spawning sites than does chemical imprinting.

Once the eggs hatch and the larval anemonefishes recognize their specific host anemone, they must then begin the process that allows them to live symbiotically among the anemone's tentacles without being harmed. This mechanism is a behavioral process known as "acclimation", through which the anemonefishes gain a mucous coating that protects them from the stinging tentacles (Fautin 1991, Elliott 1994, 1997). Through this process of acclimation, the anemonefish is initially stung by the anemone's tentacles and swims away only to return again, this time allowing more of its body to come into contact with the tentacles (Elliott 1994, Fautin 1991, Lubbock 1980). Eventually the anemonefish is able to swim freely among the tentacles without being stung (Lubbock 1980, Fautin 1991). Although most researchers agree on the acclimation process, there are conflicting theories on where the protective mucous comes from (Lubbock 1980, Elliott 1994, Mebs 1994). One theory is that the anemonefishes acquire the mucous from their host anemone during the acclimation process, and that this mucous prevents them from being stung (Elliott 1994). The other theory is that the anemonefishes themselves produce a mucous coating that lacks substances that elicit nematocyst discharge by the host anemones, therefore preventing them from being stung (Lubbock 1980, Elliott 1994). While most studies support the fact that the mucous coating is obtained from contact with the host anemone, work by Miyagawa (1989), provides evidence that supports this theory for some species of anemonefishes. This research showed that some species of anemonefishes can produce their own mucous coating and are therefore "innately" protected from certain species of anemones. It is not known whether the mucous coating resembles anemone mucous, if it contains substances that inhibit nematocyst discharge, or if it masks the anemonefishes stimuli for discharge (Elliott 1994).

ADVANTAGES OF THE RELATIONSHIP

A mutualistic symbiotic relationship like the one between anemones and anemonefishes allows both species to benefit in some way from the partnership. Although the advantages to the anemonefishes in this interaction are far more numerous than the benefits to the anemone hosts, the anemones also gain many advantages.

Advantages for the Anemone

One advantage gained by anemones, though somewhat controversial, is that the anemonefishes feed their host anemone (Fautin 1991). It has been observed that oversized food items that cannot be consumed all at once are brought to the host anemone and stuffed among the tentacles (Fautin 1991, Allen 1972). Allen (1972) suggested that the fish are merely trying to take the food item to their home and away from potential competitors rather than deliberately feeding the anemones. The frequency of this behavior is high in aquaria, but it has been observed less frequently in nature. This raises the question as to whether this advantage was significant in the evolution of the symbiotic relationship or if this behavior is mostly a result of captivity (Fautin 1991).

According to Fautin and Allen (1992), sea anemones need sulfur, nitrogen, and other chemical elements in order to grow and reproduce. Because anemones are not voracious predators and their prey consists of whatever organisms come into contact with them, the supply of these needed elements is most likely small and inconsistent. Fautin suggested that the waste products of the anemonefishes may be a much more significant and predictable nutritive contribution to the

anemones, providing them with sulfur, nitrogen, and other possible nutrients (Fautin 1991). This theory has not yet been studied scientifically and future research could be done to determine if anemonefishes waste products provide beneficial nutrients to sea anemones.

Another benefit that the anemone gains from their anemonefish symbionts is protection from other fish (Randall 2002). There are various species of fish inhabiting the coral reefs that feed on anemone tentacles and sometimes whole sea anemones (Allen 1972, Randall 2002). Anemonefishes are highly territorial of their host and defend the anemone against these predators (Arvedlund 2000). Allen (1972) observed that when an anemonefish is removed from its host anemone, these other fish would swarm in and begin to feed on the anemone. The presence of the anemonefishes, therefore, provides protection to the anemone from these coelenterate-feeding fishes (Allen 1972).

There have been numerous other benefits to the anemones hypothesized by different researchers studying this symbiotic relationship. Some of these benefits include theories that the anemonefishes rid the anemones of parasites and infected tissues (Perry 1983, Allen 1972), that anemonefishes may serve as an attractant to other fish which become prey for the anemones (Perry 1983), and that the tactile stimulation caused by the anemonefishes swimming among the tentacles is beneficial and needed by the anemones (Allen 1972). None of these theories have been studied or proved scientifically and are disagreed upon by different researchers. Scientific research needs to be conducted in order to know for sure whether these are definite benefits provided to the anemones by their symbiotic relationship with the anemonefishes.

Advantages to the Anemonefishes

As stated previously, the anemonefishes gain the significant advantages of this relationship. The main advantage that *Amphiprion* gains is that of protection (Fautin 1991, Buston 2003). The stinging tentacles of the anemones provide a safe haven from predators for the anemonefishes (Fautin 1991, Allen 1972, Buston 2003). While during the day anemonefishes may venture short distances away from the anemone to find food, at night the fishes settle deep into the tentacles and remain there until the morning (Fautin 1991). It has also been observed that the smaller the *Amphiprion*, the closer the relationship with the anemone, sometimes to the point of rarely leaving the confines of the tentacles (Allen 1972). This protection is not only beneficial to adult anemonefishes, but to eggs as well (Buston 2003). As stated earlier in the paper, adult anemonefishes select spawning sites which position the eggs near the anemone and in contact with the tentacles (Arvedlund 2000). Although anemonefishes are very territorial and protective of their eggs, this position would provide extra protection from predators that did not want to come into contact with the tentacles (Fautin 1991, Elliott 1995).

Another benefit is that *Amphiprion* may gain some nutrients from feeding on waste material from the anemone or even on the anemones tentacles (Fautin 1991, Allen 1972). Upon examination of certain species of *Amphiprion*, researchers found nematocysts and large amounts of zooanthellae, which is symbiotic in anemone tentacles (Allen 1972). This indicates that the anemonefishes do in fact ingest at least small amounts of the tentacles; however whether this behavior provides part of its nourishment or serves another purpose needs to be researched further.

According to Allen (1972), another possible benefit to the anemonefishes is that the stinging tentacles of the anemones remove external parasites from the fishes. It has been observed that fishes in a captive environment void of their host anemones are more prone to aquarium diseases and parasites. However, conflicting research has found parasitic isopods on certain species of anemonefishes living with anemones, making the need for more research in this area a necessity (Allen 1972). One final benefit that has been hypothesized is that the tactile stimulation that the fishes receive from the tentacles of the anemone may be beneficial to their health and well being (Fautin 1991, Allen 1972).

Is the Mutualism Necessary for Survival?

These two organisms both receive multiple benefits from their symbiotic relationship, but is the relationship actually necessary for survival? On the anemonefishes part, the answer is yes. These species of fish rarely occur in nature without being associated with a host anemone, and in experiments removing either the anemonefish from the anemone or vice versa, the anemonefishes almost always became prey for larger fish (Allen 1972, Fautin 1991, Mariscal 1970). Anemones, on the other hand, are often found in nature without anemonefishes, thereby supporting the hypothesis that the fishes are not essential to the anemone for survival; however provide them with some useful benefits (Fautin 1991, Allen 1972, Elliott 1995).

CONFLICTING IDEAS AND FUTURE RESEARCH

In the course of conducting research for this paper, I encountered much conflicting research. These inconsistencies could result from a variety of reasons. One of these reasons could be different researchers' definitions of terms. For example, I noticed that authors define "acclimation" differently. Another reason for inconsistent data may be that different species interact differently with each other. For example, one researcher could have observed a behavior between one certain species of anemonefish and host anemone and then generalized that the behavior takes place in all species. Another researcher could then state that they did not observe this behavior in a certain other species and this leads to contradictory data when in fact, both researchers may be correct. Certain species of anemonefishes may interact differently with their host anemone than another species does with its anemone. Therefore future research should be conducted specifically on certain species and generalization should not be made about all fish in the *Amphiprion* genus and their host anemones from observing only certain species. Another possible reason for varying results is the way that research was conducted. Most of the experiments conducted using anemonefish and sea anemones were done in captive conditions with certain observations made in captivity being inconsistent with observations made in nature. I believe that these inconsistencies were due to the captive conditions. Anemonefishes in captivity will most likely display certain unnatural behaviors from the result of being in a captive environment and therefore it may be hard to tell whether a certain result would also be obtained in nature. For example, Mariscal (1970) observed that when kept in captivity in the absence of a sea anemone, anemonefishes will bathe among air bubbles or vegetation. This behavior is not observed in nature, however, because the anemonefishes are always associated with a host anemone. Another behavior that has been observed mainly in captive conditions is the feeding of the anemone by its anemonefish (Fautin 1991). Allen (1972) only observed this behavior twice in nature and hypothesized that this behavior is induced by captive conditions. Most of these

experiments are conducted in captive environments because it is easier to manipulate the subjects and experimental variables than would be in nature, plus it is easier to observe the subjects for extended periods of time when they are easily accessible in captivity. However, in order to obtain more accurate, consistent results, future research should be more focused on experiments and observations in nature. One way to accomplish this could be to use video recorders to observe interactions between anemonefishes and sea anemones in nature. Jonsson et al. (2001) used remotely operated vehicles attached with high-quality cameras to observe the interactions of different species of shrimp with anthozoans. This same method could be used to study anemonefishes and sea anemones without researchers having to spend immense amounts of time underwater and reducing the possibility of distracting the fishes from their normal behavior. Using this technique, researchers could determine answers to a variety of questions that have not been studied in-depth in natural conditions. For example, it could be determined if the acclimation process takes place the same way in nature as it does in captive conditions. It could also be used to determine if anemonefishes interact the same way with their host anemones as they do in captivity, for example, feeding their anemone. Another possible question that could be studied by using this method is if anemonefishes kept in nature interact and live symbiotically with the same anemone species that they live with in captivity. For example, do anemonefishes live with certain species of anemones in nature that they do not live with in captivity, or do they live with certain species in captivity that they are not found associating with in nature.

Some other topics for future research could involve experiments to determine the mechanism of the mucous coating that some species of anemonefishes produce themselves. Experiments could be done to determine if the mucous is similar to anemone mucous, therefore preventing nematocyst discharge or if the mucous contains some other compounds that prevent nematocysts from firing. First, the chemical composition of both the anemone mucous and the protective mucous found on anemonefishes would have to be determined. The chemicals derived from both would then have to be studied to determine if the anemonefishes mucous contained the same compounds as those found in anemone mucous, and if these compounds prevent nematocyst firing. If the chemical compounds in the mucous from anemonefishes are found to be different than those found in the mucous obtained from the anemones, these compounds would need to be studied to determine their mechanism of protection from nematocysts. Another interesting topic for future research would be to see if other species of fish that associate with sea anemones acquire protection as do the anemonefishes, or if they still use the anemone for protection while occasionally getting stung. Randall and Fautin (2002) conducted research on other fishes that associate with sea anemones, such as, cardinalfishes (Apogonidae), wrasses (Labridae), butterflyfishes (Chaetodontidae), and hawkfishes (Cirrhitidae). However, there have not been any experiments done comparing the relationships of these fishes with sea anemones to the relationship of members of the genus *Amphiprion* and sea anemones. The symbiotic relationship between anemonefishes and sea anemones encompasses many aspects that are still unclear and controversial or beginning to be researched. There are a wide variety of possibilities for future research and experimentation relating both directly and indirectly to this relationship.

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