

THE ADVANTAGE OF JUVENILE COLORATION
IN REEF FISHES

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Abstract

Juvenile reef fishes often have a color pattern different from that of adults. It has been theorized that this reduces the aggression received by juveniles from adult conspecifics. This was tested using two species of *Labroides* cleaning wrasses in which certain-sized individuals can quickly shift back and forth between the adult and juvenile color patterns. Adult *Labroides phthirophagus* has the same single-male grouping social structure as previously described for *L. dimidiatus*. Small *L. phthirophagus* and *L. dimidiatus* in juvenile coloration shifted to adult coloration when isolated and then quickly shifted back to juvenile coloration when chased by an adult conspecific female. In *L. phthirophagus* the adult females attacked small cleaners more frequently when they displayed the adult color pattern, indicating that juvenile coloration gives some protection from conspecific aggression. Two other species of labrids, *Thalassoma duperrey* and *Coris gaimard*, showed the ability to shift back to juvenile coloration when aggression was received from conspecific adults, although the shift was not nearly as rapid as seen in *Labroides* species.

Dascyllus albisella and *Zebrasoma flavescens*, common reef fishes, preferred to solicit cleaning (by posing) from the adult-colored *L. phthirophagus*, indicating that some hosts prefer the adult color pattern. Small *L. phthirophagus* shifted to adult coloration more quickly when starved than when provided with host fish on which to feed, indicating that the coloration shift is motivated by hunger.

Even though juvenile coloration in some fishes may reduce the aggression received from adults, in cleaner wrasses it also reduces food availability, making it advantageous for them to shift to adult-coloration as soon as possible. Cleaner wrasses have developed a quick, reversible coloration shift that allows changing to adult coloration at a small size but allows reversing coloration if too much aggression is received.

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Chapter 1

Introduction

Juvenile coral reef fishes often have a color pattern different from that of the adults. This is found in numerous families encompassing several feeding guilds (Thresher, 1984). Some species have juvenile color patterns so different from the adult pattern that they were once described as different species (Randall, 1996). These striking color changes, which in most species are slow and gradual, have been labeled ontogenetic color changes (OCC) by Booth (1990). Crook (1997) described any quick and easily reversible color change as physiological color patterns (PCP).

The use of PCPs to signal dominance, aggression or subordination has been found in several species. Magnuson and Gooding (1971) described pilotfish (*Naucrates ductor*, generally found associated with sharks) shifting from the usual barred pattern to a non-barred pattern when chasing conspecifics that are associated with another shark. Baerends *et al.* (1986) showed that the cichlid fish *Chromidotilapia guntheri* displays a pattern of vertical bars when displaying aggression and adopts a horizontal stripe pattern when being chased. Beeching (1995) demonstrated that the cichlid fish *Astronotus ocellatus* darkens from olive green to brownish black when defeated in combat, and this dark coloration reduced aggression from conspecifics. Crook (1997) showed that in juvenile bullethead parrotfish (*Chlorurus sordidus*) certain PCPs were correlated with a larger body size, possibly indicating dominance. Crook (1997) also showed that PCPs might be used for anti-predator purposes and to facilitate schooling.

PCPs are used by animals other than fishes. Cephalopods are well known for their rapid coloration changes (Demski, 1992). Reptiles are also able to show quick coloration shifts. The male Anolis lizard darkens in color after losing a fight with another male (Summers and Greenberg, 1995). The mountain spiny lizard, *Sceloporus jarrovi*, also

shows a quick darkening in coloration, but this may be more for thermoregulation (Sherbrooke *et al.* 1994).

OCCs are also a factor in dominance relationships. Barlow (1976) showed that Midas cichlids start out in the 'normal' coloration of gray and black with patches of red and yellow. Sometime between 3 months and several years of age a small percentage (about 7-10%) lose the dark coloration and display various shades of red through yellow, called the 'gold' coloration. Juvenile fish displaying the gold coloration were dominant in groups of conspecific juveniles (Barlow and Ballin, 1976) and grew at a faster rate when reared with other 'normal' colored juveniles (Barlow, 1973). Dickman *et al.* (1988) demonstrated that the coloration change in this species is caused by the death and removal of the melanophores that produce the dark coloration.

Another situation where OCCs affect intraspecific interactions is in the cichlid *Pelvicachromis pulcher* (Martin and Taborsky, 1997). The fish differentiate into red and yellow color morphs at about 6 months of age. Males use different mating tactics – pair mating, harem mating, and satellite mating. All of the satellite males were the yellow morph, and all the harem males were the red morph, resulting in a higher spawning success for the red-morph males.

There has been some documentation of coloration changes that occur in a time frame between OCCs (once or twice a lifetime) and PCPs (in minutes or less). The giant kelpfish, *Heterostichus rostratus*, shifts between brown, green, and red morphs over a time frame of several weeks, assumedly to match the adjoining plants (Stepien, 1987).

There are several hypotheses accounting for distinct juvenile coloration in animals. One hypothesis is to minimize aggression from conspecifics that pose the greatest threat to an adult. A new recruit would benefit from settling in an area near adults, as this would ensure the presence of food and mates (for when it eventually matures). If the recruit has the same coloration as the adults then settling near them would entail some risk, as a

territorial adult would act aggressively toward a similarly colored animal, treating it as a high-risk intruder into its territory. An intruder with the same coloration and size as the resident would most likely have the greatest niche overlap and its presence would probably result in the resident getting less food or fewer mates. A differently colored juvenile would pose a lesser threat and thus receive lower levels of aggression from the resident. Juvenile color pattern could possibly reduce the aggression received from larger conspecifics in a way similar to the delayed plumage maturation seen in young male passerine birds (Rohwer et al., 1980 and Flood, 1984), where first year males exhibit the duller female plumage even though they are at a reproductive age. Delayed plumage maturation is generally seen in male birds, but it has been documented in females as well (Stutchbury and Robertson, 1987, Morton, 1990).

Several studies indicate that a juvenile fish's coloration can affect the amount of aggression received, but the mechanism appears to have some variability. Fricke (1980) found that adults attacked models with an adult color pattern more often than models with a juvenile color pattern in the Emperor Angelfish, *Pomacanthus imperator*. In contrast, Neal (1993) found that adult Garibaldi, *Hypsypops rubicundus*, attacked small fish with the juvenile color pattern more often than it attacked those with the adult color pattern. Results similar to Neal's were found by Koebele (unpublished), who saw more attacks on the juvenile coloration in the Sea of Cortez damselfish, *Eupomacentrus rectifraenum*. Unfortunately these three studies all had small sample sizes.

The effect of adults on juvenile recruitment in species in which juveniles have nearly the same color pattern as the adults is also variable. Sale (1976) showed that juvenile *Pomacentrus wardi* had a higher recruitment and survival rate when adults were removed from the area, whereas Sweatman (1983) showed that juveniles of two *Dascyllus* species recruited more heavily to coral heads that had conspecific adults. The general trend appears to be that species that defend individual territories will attack

juveniles more than adults, whereas other species attack juveniles less. In either case, the juvenile coloration changes how the smaller fish is treated by larger conspecifics.

An alternate hypothesis for juvenile coloration of some species could be that it increases food availability for fish that 'clean' others. Cleaning is a phenomenon seen regularly in reef fishes where a fish or shrimp will remove ectoparasites and mucus from the body of host fish. Many fishes clean only when they are juveniles (Thresher, 1980), and may use unique color as an easily learned stimulus to indicate to the host fish that it will be cleaned. For fishes that also clean as adults, hosts could prefer the different juvenile coloration. This could possibly be due to less frequent or less painful bites taken by juveniles, and would serve to increase the number of hosts available to the juvenile.

A third possible reason for juvenile coloration is to camouflage the vulnerable juvenile as the larger adult is at a much smaller risk from predation. Many aspects of fish coloration have been attributed to camouflage and predator avoidance (Neudecker, 1989, Thresher, 1984). A theory that combines the cleaning and camouflage theories is that a fish that cleans only as a juvenile needs an obvious coloration for cleaning but when larger would benefit from a more cryptic coloration.

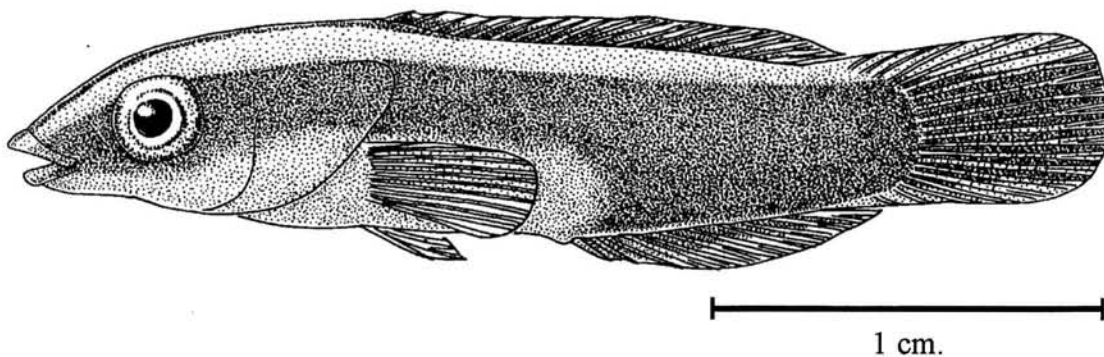
The Hawaiian cleaner wrasse, *Labroides phthirophagus* (Randall, 1958), was used to test these hypotheses. It is a small fish (up to 10 cm TL) endemic to the Hawaiian Islands. Both adult- and juvenile-colored individuals occupy 'cleaning stations' on the reef where other fishes come to have parasites and mucus removed. It is an obligate cleaner, as it has not been seen eating anything else when in a natural setting. This species is monandric and protogynous, in that all small cleaners are females and all males are sex-changed from females (Youngbluth, 1968). Juveniles show a color pattern that is different from the adults (a dark blue occupies the yellow areas shown by adults) but have some similarities (both have a black horizontal band through the middle of the fish - see Figures 1 and 2). The shift to the adult pattern usually occurs at a size of about 3 to 4 cm.

Both male and female adults show the same color pattern. Single males occur in groups that contain multiple females, including juveniles. Sexual maturity as a female is reached at about 5.5 cm TL and sex change to male occurs at a size of about 6.5 cm TL or larger (Youngbluth, 1966).

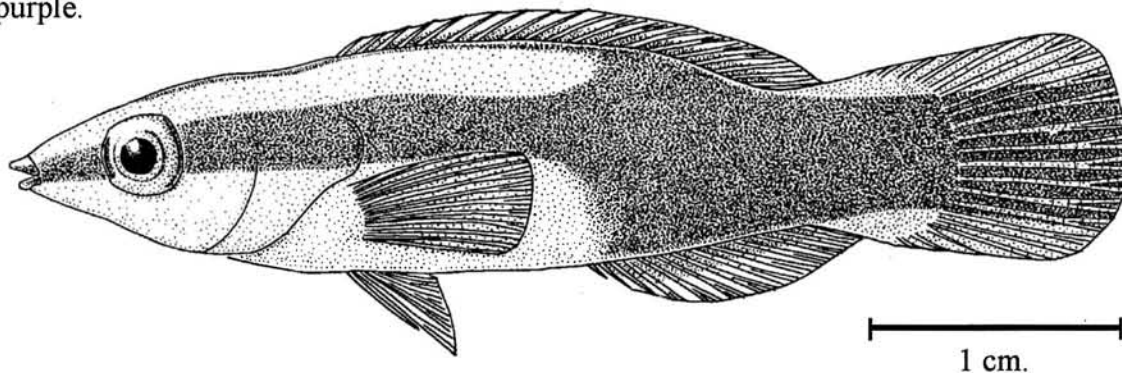
Much research has been done on cleaning symbiosis (for a review see Losey, 1987 or Losey *et al.*, in review). Studies indicate that cleaners give tactile stimulation to the host fish by moving their pectoral fins across the side of the host fish while the cleaner is inspecting for parasites or scooping mucus. In cleaner shrimp, the antennae are used to tactically stimulate the host fish. This apparently motivates the host fish to return to the cleaning station, regardless of the parasite load on the host fish. Lenke (1982, 1987, and 1988) has indicated the possible importance of endocrine control in the cleaning behavior of *L. dimidiatus* by using injections of neurohormones to decrease the time exhibiting cleaning behavior. The relationship between cleaner and host could range from mutualistic (harmful parasites are removed) to parasitic (only mucus is removed) and is dependent on the relative densities of the hosts and cleaners (Addicot 1984).

The Hawaiian Cleaning Wrasse, *L. phthirophagus*, is dependent on host fishes coming to the cleaning station, and the preference of these host fishes for a particular cleaner coloration could be a significant factor in the ability of cleaners to obtain sufficient food. It is suspected that most hosts learn to visit cleaners (Losey and Margules, 1974; Losey, 1979), but recent research has shown that some hosts will pose the first time they see a cleaner (Losey *et al.*, 1995). Regardless of how the posing starts, learning should affect the host's preference for cleaner coloration and would be modified by subsequent encounters with cleaners. Around Oahu the adult cleaners are generally much more common than the juveniles, so any preference for the adults could be a result of the host preferring the color pattern it has encountered most frequently.

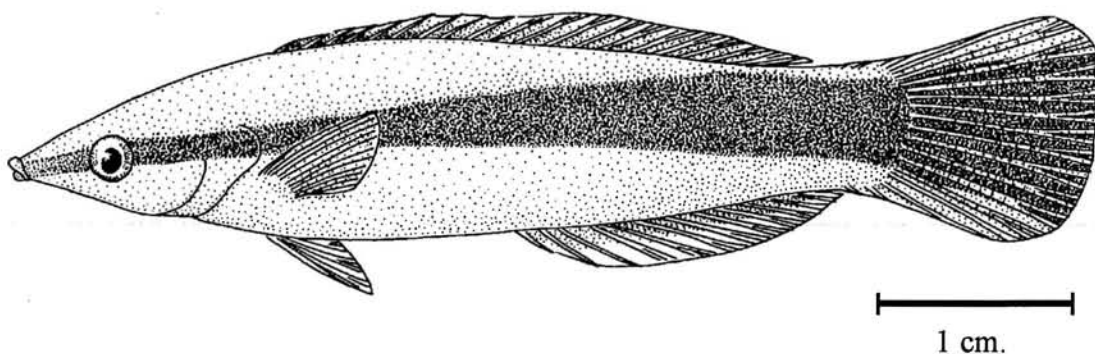
A. Juvenile of *L. phthirophagus* or *L. dimidiatus*. Dorsal stripe is medium blue to purple. Rest of body is black with a grayish area on the ventral side.



B. Adult *L. phthirophagus*. Stripe down side is black. Anterior half of body is bright yellow. Dorsal and anal fins are light blue. Dorsal and ventral areas of tail are light purple.



C. Adult *L. dimidiatus*. Stripe down side is black. Dorsal and ventral parts of body are pale yellow to white anteriorly and blending to light blue posteriorly.



Illustrations by S. G. Monden.

Figure 1. Color patterns of *Labroides* species used.

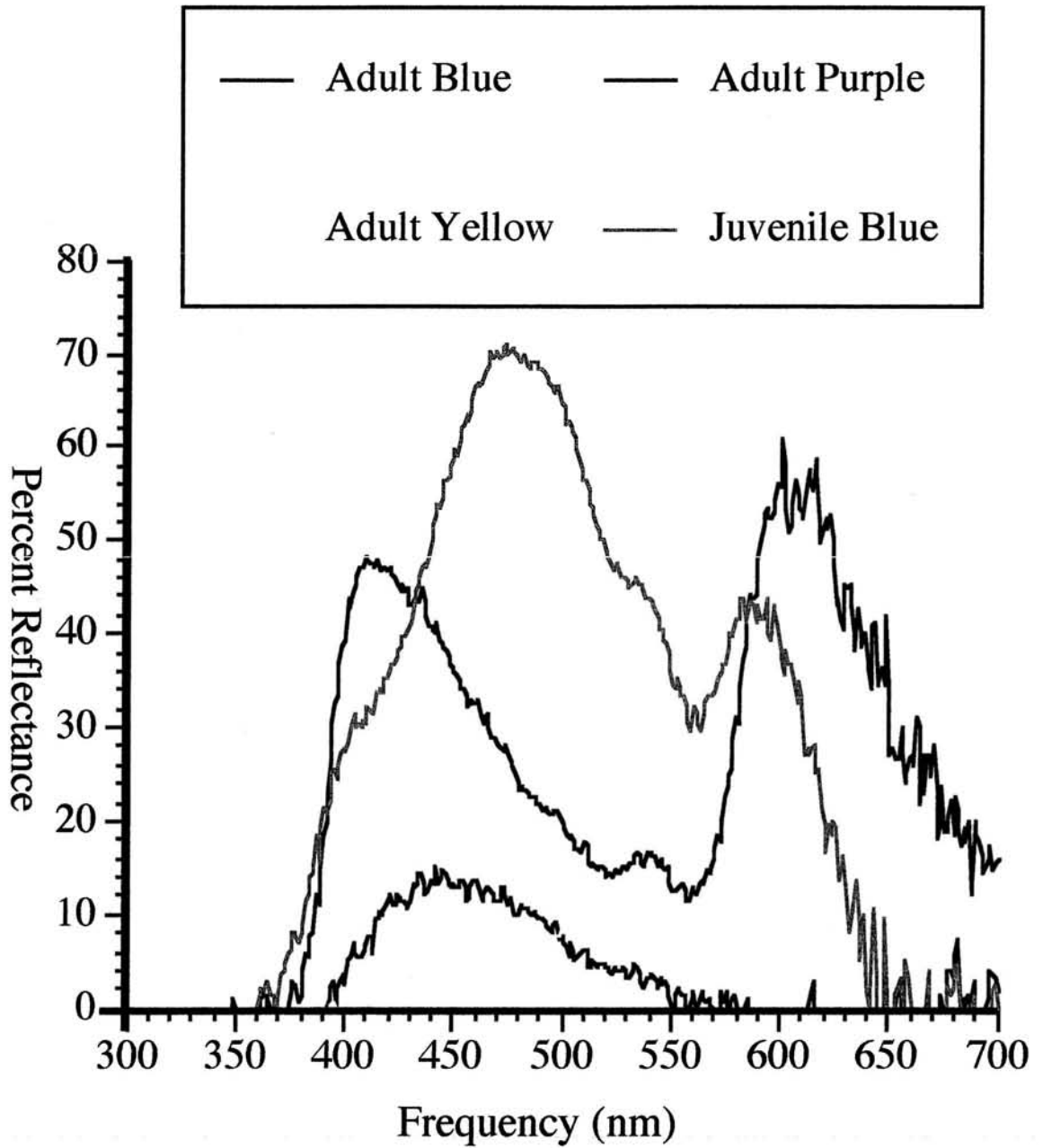


Figure 2. Reflectance Spectrum for colors on *Labroides phthirophagus*

Chapter 2

Social Structure

Introduction

The social organization of *Labroides phthirophagus* has not been described in detail (see Youngbluth, 1968), but that of a cogener, *L. dimidiatus*, was closely examined by Robertson (1974) and Kuwamura (1984) and serves as a baseline. Adult *L. dimidiatus* have a different color pattern than adult *L. phthirophagus* but the juvenile coloration is almost identical [see Randall (1958) for a full description]. *L. dimidiatus* is found throughout the tropical Western Pacific and Indian Oceans and is a facultative cleaner, as it feeds in part on benthic and pelagic organisms (Losey, 1974a, Kuwamura, 1984). It is also a monandric protogynous species that forms social groups of one male and 1-12 females (Robertson, 1974). This social system is similar to that found in many labrids (Tribble, 1982, Warner, 1984 and Hoffman, 1985). The territory of the male includes almost all of the home ranges of the females, and he frequently (at least once an hour) visits each female in the group (Robertson, 1974). Males are aggressive toward all females in the group to prevent them from changing sex, and females show aggression toward significantly smaller females in the group (Kuwamura, 1984). The detailed ethogram for *L. dimidiatus* provided by Robertson (1974) closely matched the ethogram of the cleaning and spawning behavior of *L. phthirophagus* as described by Youngbluth (1968).

A study was performed to determine the social structure of *L. phthirophagus* and compare it to that of *L. dimidiatus* as described by Robertson (1974) and Kuwamura (1984). Differences between the two species' social structure may be due to *L. phthirophagus*' being an obligate cleaner (Youngbluth, 1968).

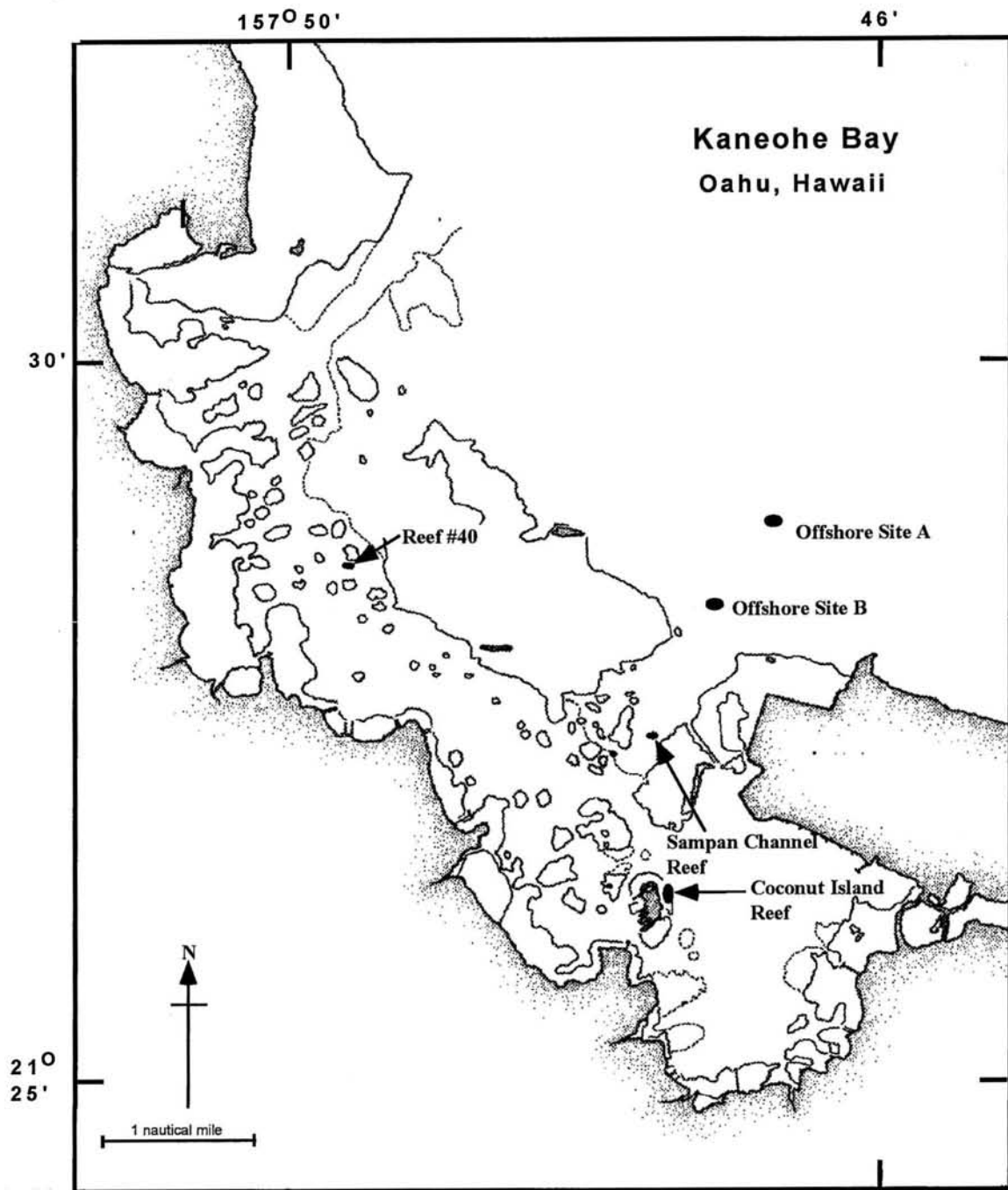


Figure 3. Locations of study sites

The effect of a juvenile on the other members of the social group was also investigated to answer some questions that developed as a result of experiments in subsequent chapters.

Methods

Unless otherwise noted, all statistics in this dissertation were calculated according to Sokal and Rohlf (1981).

General

A small patch reef in Kaneohe Bay (reef #40) was used as the main study site (Figures 3 and 4), and observations were subsequently made offshore of Kaneohe Bay (offshore site A, Figures 3 and 5) to use for comparison. A later short-term study (2 months) was performed at three different sites (Figure 3) in the same time period - Coconut Island reef (inside Kaneohe Bay), Sampan channel reef (at the entrance to Kaneohe Bay), and a second offshore site (offshore site B). The isolated coral reefs in Kaneohe Bay are very convenient and useful for long-term studies, but the structure and layout of these reefs are unique. Almost all of the reefs in Hawaii are continuous or at most separated by sand or rubble at approximately the same depth, and not the deeper silt channels that are found in Kaneohe Bay (personal observation). The offshore sites have continuous spur-and-groove rocky reefs typical of most of Hawaii.

A census was made at reef #40 approximately 1-2 times per week for 12 months (August 1989 – August 1990) to determine site attachment of individuals and to observe social behavior (e.g. movement of males to cleaning stations occupied by females). Interactions were defined as any observation where two or more cleaner were within 50 cm of each other. Offshore observations were later made about once per week for four months (September 1990 – January 1991), also looking at site attachment and social

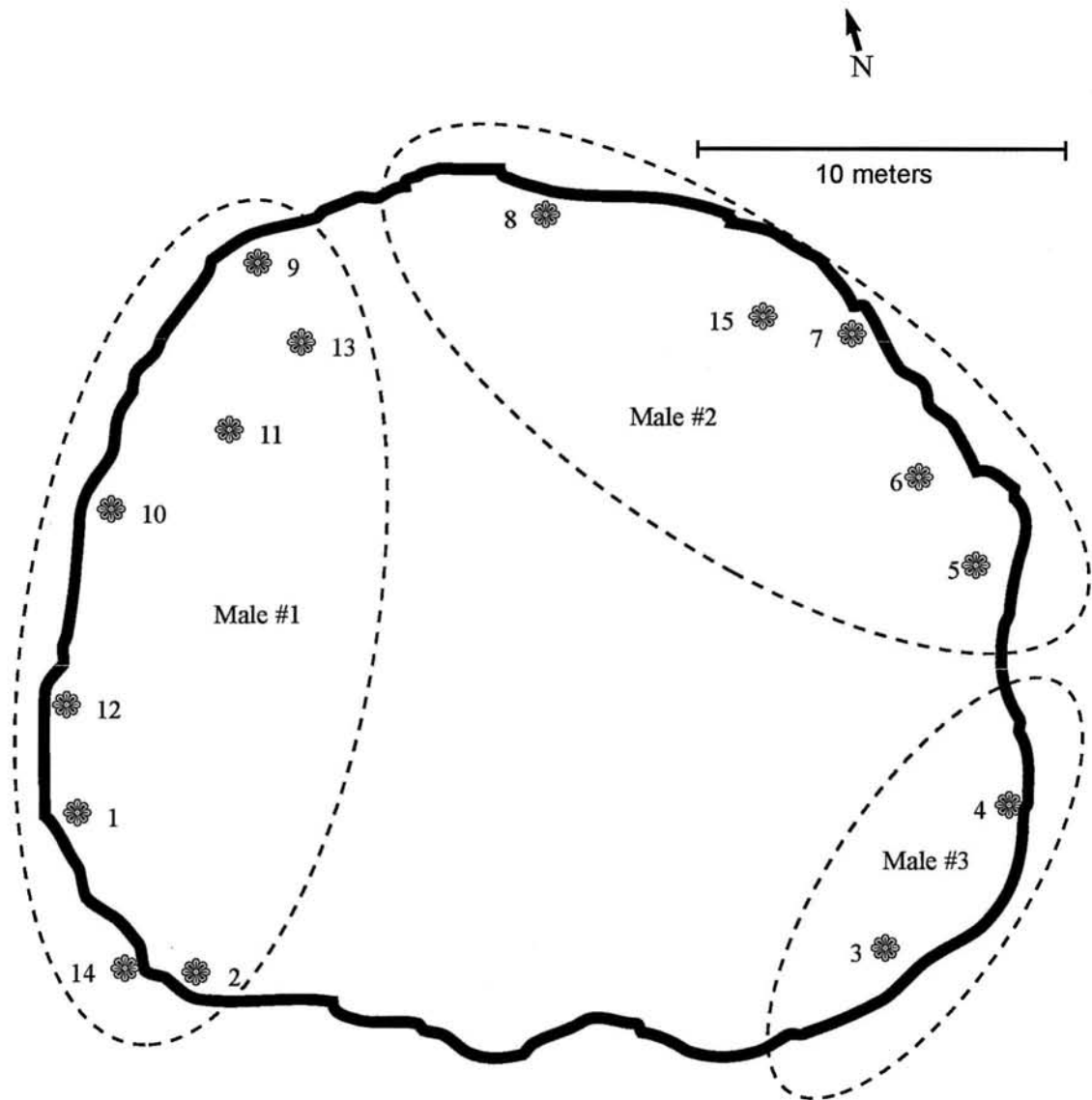


Figure 4. Reef #40 in Kaneohe Bay

Cleaning stations indicated by a ☼. Dashed lines show male's territory.

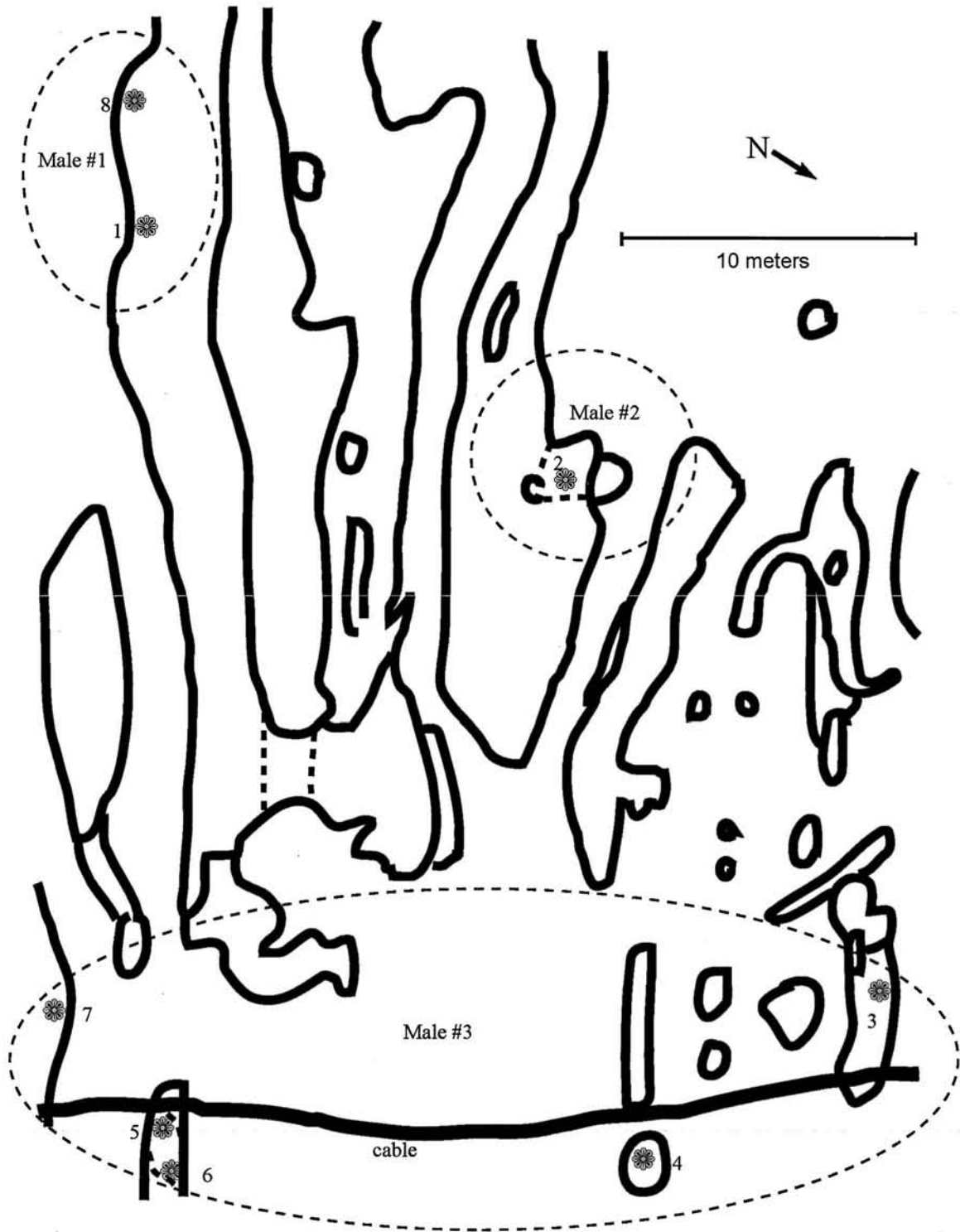


Figure 5. Offshore Site A

Cleaning stations indicated by a . Dashed lines show male's territory.

behavior. Observations for the short-term study were made 1-3 times per week for 2 months (June – August 1998) to look at juvenile social interactions. Kaneohe Bay observations were made using scuba or snorkel, whereas all offshore sightings used scuba. Because cleaners shelter in the reef at night, all observations were made during the day (9 AM to 6 PM). Individual fish were identified by noting variations in markings and recording them on underwater paper.

Cleaning stations in Kaneohe Bay were marked with monofilament line approximately 50 cm. in length tied to the coral at one end and a small float (plastic vials or fishing bobbers) tied to the other end. The float was numbered with a black marker. Youngbluth (1968) showed that this marking procedure did not affect the fish's behavior. This method worked well in Kaneohe Bay but the floats did not last long offshore (due to higher wave action) so stations were marked with surveying tape tied to coral rubble, and an underwater map with marked features was used for navigation. Surveying tape was also used during the short-term study.

Observations showed that *L. phthirophagus* seldom ventured outside an area with a three-meter diameter unless they were moving to another cleaning station, and thus a cleaning station was defined as an area with a three-meter diameter inside which the cleaner spent most of its time. The main exception to this occurred when a parrotfish or wrasse continued to swim while being cleaned, but in these instances the cleaner would quickly return to the cleaning station after finishing the cleaning bout. Cleaners observed in transit between cleaning stations were seen to occasionally stop for a cleaning bout but spent most of the time quickly transiting to the next station.

Sex was determined by observing the 'pass and quiver' behavior described by Youngbluth (1968), also called 'flutter-run' for *L. dimidiatus* by Robertson (1974). This behavior is only performed by the male *Labroides* in front of a female, and it consists of the male quickly swimming at the female and then turning away, spreading unpaired fins

and shaking slightly. This determination was confirmed by observing the change in abdominal distention of suspected females before and after spawning. These methods of sex determination were verified histologically by examining cleaners from other reefs that showed the same behavior or abdominal swelling.

Reef #40, Kaneohe Bay

Territoriality was tested by using a model-bottle experiment (Myrberg and Thresher, 1974). This experiment consisted of placing a male or female *L. phthirophagus* inside a clear plastic container and placing the container inside the subject's cleaning station for two minutes while watching the subject's reaction. The responses were divided into attack, display, inspect/clean, and no noticeable response. The cleaner in the bottle was caught far away from the subject's cleaning station so the chance of them meeting previously was very small. As a control, different species of wrasses were placed in the bottle and the test was repeated. The fish in the bottle was active in all trials. This test was performed at reef #40 and the sites in the short-term study.

It was desired to see if the total number of cleaners or the amount of movement seen on the reef has a cyclic pattern. Many reef fishes are known to have a lunar spawning pattern, and this may affect the placement of cleaners or the amount of movement. The recruitment of juveniles often appears to have a yearly cycle with a peak in the summer, possibly due to changes in current patterns affecting planktonic larvae or increased spawning during the spring or summer (Thresher, 1984, Lobel, 1989, and Danilowicz, 1997).

For each day of observation at reef #40 the total number of cleaners seen and movement observed was recorded (the movement of a cleaner from one station to another was counted as one movement unit). The date of the observation was converted to number of days after full moon for the lunar analysis, and to a day count starting at the

first observation for the yearly analysis. In accordance with Cryer (1986) the date number was converted to sine and cosine functions with a yearly or lunar month cycle frequency. This was then regressed with the total number of movement data.

To see if the location of some cleaning stations provided better host resources than others, observations were made at reef #40 in which a record was made of the amount of time different species of host fishes spent being cleaned. Gorlick (1980) showed that cleaners preferred certain families as host fish so it was theorized that this preference may create a hierarchy of station quality. Ten-minute observations were made with stopwatches to record the cleaning time of common families of fish (Labridae, Acanthuridae, Pomacentridae, and others). Four sets of observations were made at three different cleaning stations (stations #1, 3, and 4, see Figure 4) on 10 different days.

Offshore of Kaneohe Bay (site A)

Most of the observations in the general section above were also performed at the offshore site. The offshore study was not long enough to perform the tests for cyclic patterns. No model-bottle experiments were conducted nor were any data taken on host fish cleaned.

Short-term Study

General

This study was conducted at the end of my dissertation research to answer some questions brought up by results from experiments described in later chapters. The part of the results that described social interactions is included in this chapter. The results dealing with color change are included in Chapter 3 because they require knowledge of the results of other experiments in Chapter 3 for full understanding.

Six social groups were observed at two different sites in or near Kaneohe Bay. Four sites were on the reef surrounding Coconut Island and two were at an offshore site. The Coconut Island sites were on the east-northeast side of the island at a depth of 1-2.5 meters, and observations were made using snorkel. The offshore sites were 13-14 meters deep and scuba was used for all observations. Each social group contained one male and 2-4 females (in either adult or juvenile coloration). The study was concluded when one of the juvenile-colored cleaners shifted to adult coloration or, in one case, when the juvenile disappeared.

Adult-Juvenile Interactions and Time Budgets

It was desired to see how the presence of a juvenile cleaner affected the behavior of the social group. The other studies in this chapter contain only two observations of a juvenile-colored cleaner due to the fact that the observer was not yet aware that they usually resided in small caves and was not looking for them in these locations. Three of the social groups were picked because they contained a cleaner showing juvenile coloration, and these were selected as the treatment reefs. A nearby social group not containing a juvenile was used as the control group.

Observations were made on the number and type of social interactions between adult and juvenile cleaners, the time budgets of adult males, and the species cleaned by juveniles as compared to adult females. Twenty-minute observations were made recording data on cleaners from each social group, first the male and then a small cleaner (in juvenile coloration for each treatment reef and in adult coloration for the control reefs). Attempts were made to get cleaning times for juveniles versus adults but getting close enough to the juveniles to record these data usually scared off the host fish, biasing the data. Species were grouped by family for ease of analysis and identification.

The male's time budget was broken down into inspection/cleaning, interacting with other cleaners, transiting between stations, and other activities (usually moving between host fish or waiting for a host fish).

The data obtained were based on 40 hours of observations on the cleaners at Coconut Island Reef (four social groups) and 10 hours at the offshore B Reef (two social groups).

Model-bottle

A model-bottle experiment was conducted to confirm the results from the experiment conducted on reef #40. The experiment was conducted as described for reef #40 except that a juvenile-colored cleaner was placed in the bottle as an added treatment.

Results

Reef #40, Kaneohe Bay

A total of 551 individual sightings were recorded from 25 individual cleaners at 15 different stations. Each sighting consisted of a cleaner observed at one cleaning station (i.e., if a cleaner moved to another station, that was counted as another sighting). Cleaning stations ranged in depth from about 0.5 meters to 2.5 meters. Only four cleaners were present for the entire study, with 13 arriving during the study and 15 disappearing for unknown reasons. Males showed greater tenacity at the site as two of the three males remained for the entire study, whereas only two of the 22 females were present at both the beginning and end of observations (G-test of independence, $G_{adj} = 5.058$, $P < 0.025$, $df = 1$). Of the 13 arrivals, only one was observed displaying the juvenile color pattern. This cleaner proceeded to set up a new cleaning station and then to shift to the adult color pattern within a week. It is possible that more juveniles were

present during the study but as they tend to stay in small caves they may have been hidden from view. The other 12 arrivals (one male, 11 females) were at full-adult size (5.5 - 7.5 cm TL) when first observed, indicating that there probably was migration between patch reefs, even though the nearest reef was about 30 meters away across a 12-meter-deep silt-bottomed channel with 30-90 cm. visibility.

Each of the three males had a territory containing two to eight cleaning stations and one to six females (not all stations were continuously occupied). The males defended their territories against other males, as evidenced by model-bottle experiments and an observation where one male ventured into another male's territory and was observed by the resident male, resulting in a rapid chase. In the model-bottle experiments (see Table 1) the resident male always attacked when a male was in the bottle (three out of three trials) and performed various displays (always including the 'pass and quiver') when a female was in the bottle (three out of three trials). This indicates that cleaners can visually recognize each other's sex. Resident females always investigated the cleaner in the bottle (12 out of 12 trials) but the only obvious behavior seen was when a larger resident female would try to attack a smaller female in the jar (size difference > 0.5 cm, two out the six female-female trials). When presented with model-bottles containing other species of

Table 1. Model-bottle experiment results.

Resident cleaner	Male cleaner in bottle	Female cleaner in bottle	Host fish <i>T. duperrey</i> in bottle	Host fish <i>T. ballieui</i> in bottle
Male (N=3)	3 attacks	3 displays	3 inspections	3 inspections
Large female (N=2)	2 investigations	2 attacks	2 inspections	2 no action
Small female (N=4)	4 investigations	4 inspections	4 inspections	4 inspections

wrasses (*Thalassoma duperrey* or *T. ballieui*), almost all the resident cleaners tried to clean the wrasses (16 out of 18 trials). In the other two trials the wrasse in the bottle was ignored in favor of other host fish.

On three occasions males were observed visiting stations in other male's territories, but on only one occasion was the resident male present and he chased away the visiting male. Inside their territory, males were usually found at one cleaning station (98 out of 141 observations, designated as their primary station), but they would periodically visit all the females in their social group. The interval between visiting any single female averaged 2 hours 47 minutes for the three occasions an exact time was obtained. On six other occasions the interval was greater than two hours before the observer ran out of time and had to leave (for statistical analysis these observations were conservatively recorded as 2 hours). This is significantly greater than that seen by Robertson for *L. dimidiatus* (1974, all times < 27 min., $U_s = 99$, $P < 0.001$, Mann-Whitney U-Test, $N_1 = 9$, $N_2 = 16$).

All cleaners spent a large majority of their time at one cleaning station (68.4% of the observations), and this was labeled the cleaner's primary station. In social groups #1 and 2 a male and female shared a primary cleaning station, whereas the other social group had a separate primary station for the male. Males showed a greater amount of movement between stations, with the three males being observed moving 41 times and the 22 females moving only 28 times. Correcting for the respective number of observations (dividing moves by observations for each fish), males showed a significantly greater amount of movement [male median = 0.224 moves per observation, female median = 0.071 moves per observation, Mann-Whitney U Test, $t_s=2.25$, $P < 0.05$, $N(\text{males}) = 3$, $N(\text{females})=22$]. Females observed moving from one station to another were often visiting a male (13 out of 28 observed moves).

Table 2. Observations of times cleaners were seen at different stations, ranked in order of most to least observations.

Cleaner	Primary station	Secondary station	Tertiary station	Quaternary station	Other
Male #1	41	7	7	3	10
Male #2	37	4	4	3	1
Male #3	24	4	2	0	0
Female #1	33	18	2	0	0
Female #2	31	0	0	0	0
Female #3	20	14	1	0	0
Female #4	19	11	2	2	0
Female #5	16	2	2	1	0
Female #6	16	1	0	0	0
Female #7	15	5	0	0	0
Female #8	15	4	1	0	0
Female #9	14	4	2	1	0
Female #10	14	6	0	0	0
Female #11	13	0	0	0	0
Female #12	11	6	2	0	0
Female #13	11	4	2	2	0
Female #14	10	1	0	0	0
Female #15	8	7	0	0	0
Female #16	6	1	0	0	0
Female #17	6	1	0	0	0
Female #18	5	1	0	0	0
Female #19	4	4	1	0	0
Female #20	4	0	0	0	0
Female #21	2	1	1	0	0
Female #22	2	1	0	0	0

A male was involved in 110 out of the 112 social interactions (see Table 3). Correcting for respective number of observations, males were seen in social interactions a significantly greater percentage of the time than females [male mean = 0.413 interactions per observation, female mean = 0.121 interactions per observation, Mann-Whitney U Test, $t_s=2.06$, $P < 0.05$, $N(\text{males}) = 3$, $N(\text{females})=22$]. The few times two females were seen together (seven out of 112 observed interactions, see Table 3) there was obvious aggression (frontal chases) toward the smaller female (see mating description on next page). No interactions were seen between adult females and juveniles but this may have been a result of observations not being made in the caves and crevices where juveniles are found.

Table 3. Number of interactions seen on Reef #40

Type of interaction	Number of interactions observed
Male-Female	102
Female-Female	2
Male-Male	1
Male-Female-Female	5
Male-Juvenile	2
Total	112

For *L. phthirophagus*, the percentage of encounters that included two females (6.2%) was significantly smaller than the percentage for *L. dimidiatus* measured by Robertson (1974, 64.7%) or Kuwamura (1984, 44.8%) ($P < .005$ for both, G test of independence).

No females were observed actively moving from one social group to another. After one of the males disappeared, the one female that had been in his group was visited by, and spawned with, the neighboring male, but she stayed at the same cleaning station.

A total of ten spawnings were observed at the study site. All observed spawnings occurred within one hour of the daytime high tide. The females came to the male's cleaning station and then spawned (3 instances) in sequence or else the male swam to each of the females' cleaning stations and spawned (7 instances). When two females were present (2 instances), the larger female spawned first as predicted by the temporal threshold model of polygyny (Lutnesky and Kosaki 1995). In one instance the male was preparing to mate with a small female when a larger female arrived and chased the smaller female enough to prevent her from mating with the male (the male then mated with the larger female). The spawning behavior is described in detail in Youngbluth (1966). In summary, the male performed numerous 'passes and quivers' at the female, and then the pair performed a circular 'dance' in which the male and female circled each other several times at a distance of 4-8 cm. During the pre-spawning displays no cleaning was performed, but occasionally one of the pair would break off the displays to clean a host. After a highly variable period of these activities, the pair moved close to each other (about 2 cm apart) and slowly rose to a point about 30 centimeters above the reef, then quickly darted upward to about 60 centimeters above the reef and released gametes. The pair then quickly returned to the reef surface. The cloud of released sperm sometimes could be observed, depending on water visibility.

The analysis to look for cyclic patterns on reef #40 used the same data set as in the other analyses from this chapter (551 observations and 69 moves). It showed a yearly pattern for both movement (a cleaner moving from one cleaning station to another) and number of fish seen during surveys, whereas a lunar pattern was seen only for number of fish. The lunar pattern showed a peak at seven days after full moon, with the yearly peak

occurring in mid November. The amount of movement ranged from zero to 11 moves/observation period and the number of cleaners seen ranged from six to 13. An ANOVA was performed on each of the regressions.

Table 4. Results of cyclic pattern analysis at Reef #40

Parameter Analyzed	ANOVA Results	Significance
Yearly Movement	F = 8.65	P = 0.0007
Yearly # of Fish	F = 4.17	P = 0.0218
Lunar Movement	F = 2.38	P = 0.1037
Lunar # of Fish	F = 3.56	P = 0.0367

Offshore of Kaneohe Bay (site A)

Offshore observations generally agreed with the results from inside Kaneohe Bay, but the shorter length of the study produced smaller numbers and less significant results. Over the 4-month observation period 122 individual observations were recorded from 11 individual cleaners at eight different stations. Cleaning stations ranged in depth from about 13 meters to 15 meters. Seven cleaners were present for the entire study, with two arriving during the study and three disappearing for unknown reasons. Males showed a tendency for greater tenacity at the site, as all three males remained for the entire study. Only four of the eight females were present at both the beginning and end of observations, but the two sexes were not significantly different (G-test of independence, $G_{adj} = 2.776$, $0.1 > p > 0.05$, $N = 11$). No juveniles were observed at the site. Two spawnings were observed and the behavior matched the spawnings seen inside Kaneohe Bay.

When comparing how many cleaners stayed at each of the site for the first four months of each study (bay versus offshore site tenacity), no significant difference was seen [G-test of independence, $G_{adj} = 0.95$ (females), $G_{adj} = 1.04$ (males), $p > 0.1$ for both, $N(\text{females}) = 30$, $N(\text{males}) = 6$].

Short-term Study

There was no significant difference between males with and without juveniles in their social group for any of the activity categories (Mann Whitney U-Test, $C = 5$, $P \geq 0.1$, $N = 6$, see Table 5).

Table 5. Time budgets for short-term study.

Activity	Male w/juvenile	Male w/out juvenile
Inspection/cleaning	52.3%	57.4%
Interacting w/conspecifics	3.1%	1.8%
Transiting between stations	5.3%	6.7%
Other (not used in statistical analysis)	39.3%	34.1%

Visits by a male to a juvenile in the social group tended to be less frequent than the visits to an adult-colored female (male visiting juvenile - once every 4.4 hours, male visiting adult female - once every 3.1 hours), but the results were not significant (Mann-Whitney U-Test, $C = 15$, $P = 0.1$, $N(\text{juveniles}) = 3$, $N(\text{adults}) = 6$). Adult females were seen visiting the juvenile-colored cleaners only twice.

There were a number of families that were cleaned by only the juvenile-colored cleaner or only adult-colored cleaners. Table 6 lists the families cleaned and the number of times an individual was seen being cleaned.

Table 6. Families cleaned by the juvenile-colored and adult-colored cleaners at the two different sites, and the number of times they were observed being cleaned.

Family being cleaned	Coconut Isl. adult	Offshore B adult	Coconut Isl. juv.	Offshore B juv.
Holocentridae (squirrelfishes and soldierfishes)	0	0	24	51
Cirrihitidae (hawkfishes)	0	0	0	32
Serranidae (groupers, basslets, and anthias)	0	0	0	11
Scorpaenidae (scorpionfishes)	0	0	0	38
Muraenidae (moray eels)	0	0	14	83
Apogonidae (cardinalfishes)	0	0	62	32
Pomacanthidae (angelfishes)	0	0	0	11
Zanclidae (moorish idol)	0	62	0	0
Lutjanidae (snappers)	38	0	0	0
Labridae (wrasses)	1348	543	31	13
Chaetodontidae (butterflyfishes)	931	463	6	19
Scaridae (parrotfishes)	270	52	43	0
Pomacentridae (damselfishes)	220	477	328	139
Tetradontidae (puffers)	26	14	155	56
Ostraciidae (trunkfishes)	0	15	0	39
Acanthuridae (surgeonfishes)	206	88	40	9

Discussion

The research conducted on reef #40 and offshore of Kaneohe Bay indicates that *Labroides phthirophagus* and *L. dimidiatus* have a similar social structure, in which the females are spread out within a male's territory and the male periodically visits each of the females. *Labroides phthirophagus* females spend most of their time at one cleaning station (79%), whereas *L. dimidiatus* females roam around a significant portion of the male's territory (1/3 or 1/2 for large females - Robertson, 1974). This may be a factor in the small number of female-female encounters observed in *L. phthirophagus*. Another observed difference was that male *L. phthirophagus* on several occasions went longer than two hours without visiting the females in the social group whereas male *L. dimidiatus* were never observed waiting more than 27 minutes between visits to each female (Robertson, 1974). Robertson (1974) also observed dominant females showing male behavior in as little as 53 minutes after the male was removed. Females showing male behavior quickly changed into males and assumed control of a group of females. This emphasizes the importance of frequent visits for male *L. dimidiatus*.

These differences between the two species may stem from the fact that *L. phthirophagus* is an obligate cleaner and needs to spend a larger portion of its time at a cleaning station as opposed to travelling. Lack of vagility may provide sufficient nutrition and ensure an adequate supply of host fish that are trained to return to the reliable cleaning station. Female *L. phthirophagus* must have a higher threshold for changing sex than does *L. dimidiatus*.

The sudden appearance and disappearance of adult *L. phthirophagus* is a rather interesting phenomenon. The disappearances of the cleaners could be explained by mortality, but this would not explain the appearances. On the patch reef inside the bay there are many deep crevices in which a cleaner could hide and avoid detection, but this

would be unusual behavior for adult cleaners who are almost always seen above the reef during the daytime, and are not startled by the approach of divers. It seems unlikely that a cleaner would hide on a patch reef for long periods (several months) and then suddenly appear and start occupying a cleaning station. The only logical conclusion is that the cleaners migrate between the different patch reefs. Brock et al. (1979) showed that in Kaneohe Bay adult reef fish from adjacent patch reefs migrated to a patch reef in which all fish had been removed. Apparently this happens between populated reefs, even though this would appear to be a dangerous trip for the small wrasse as it entails swimming through open water or along the silt bottom which has little cover and is populated by many predators such as small hammerhead sharks (*Sphyrna lewini*), jacks (i.e., *Caranx melampygus*) and lizardfish (*Saurida* and *Synodus* species) (Randall, 1996). It is possible that the cleaner wrasse's 'immunity' from predation applies to these situations but the very low visibility at the bottom of these channels would make any visual identification difficult. The movement of cleaners at the offshore site is less surprising because the trip would be along terrain similar to that normally occupied by cleaners. This behavior may be maladaptive in Kaneohe Bay, but since the patch reefs seen in Kaneohe Bay make up a very small percentage of Hawaii's reefs, the danger from the movement between patch reefs may not produce a strong enough selection pressure to eliminate this behavior. It is unclear why this behavior is different from what was observed in *L. dimidiatus*.

In the analysis of cyclic patterns on reef #40, the yearly patterns may only apply to the single year sampled. Assuming that the results are representative of yearly patterns, the cycle in number of fish seen could indicate that the pulse of juveniles seen in the summer (personal observation) could be growing up to become adults by winter (the peak of the data). The yearly cycle of movement could be a side effect of the change in the number of fish, causing new females to look around for good cleaning stations and

causing males to visit these new females in order to obtain them for their social group. Further data should be obtained to substantiate these conclusions.

The results showing a lunar cycle in the number of fish are more reliable because the data cover several lunar cycles. The peak of the number of fish seen was about seven days after full moon. The meaning of this is not obvious, and the most reasonable explanation is that females are more visible at certain parts of the lunar cycle to increase food intake for spawning or to be more available for spawning with males. The females may hide or travel more during other parts of the lunar cycle and may not be as visible. These explanations are not very complete and more detailed observational data should be made to ascertain the exact reason for this cycle.

The results from the short-term study that described host posing may have been slightly biased by the presence of the observer. The location of juveniles in small caves made it difficult to observe cleaning without causing some impact. The observer stayed as far away from the station as possible but some of the more shy species (such as *Centropyge potteri*) may have been scared away. The observer tried to keep the distance constant between juvenile and adult observations so the bias would affect both samples, but the species list may be a little inaccurate as a result. Video cameras were tried in an attempt to avoid this disturbance but the limited viewing angle made this method unsatisfactory.

The visit rate was a statistic in which it appears that there was a difference but the small sample size prevented a significant result. The males appeared to visit the juvenile-colored cleaners less but it appears that the noise in the data prevented this from showing. The time budgets do not appear to be affected by the presence of a juvenile-colored cleaner, and it is believed that a larger sample size would not change this result. The cleaners seem to be unaffected by the observer and it is not believed that any bias was caused by the observer.

Chapter 3

Color Shift Experiments

Introduction

Observations of small *L. phthiophagus* show that for some period of the cleaner's development it can shift back and forth between the adult and juvenile color patterns (personal observation - see Figure 1 for a description of the different colorations). This was first observed during early attempts to catch small cleaners when a cleaner initially seen in adult coloration would end up, after a prolonged chase, showing juvenile coloration. The cleaner was placed in the catch bucket and when observed 20-30 minutes later, was back in adult coloration. Pilot experiments were conducted in which small *L. phthiophagus* in both colorations were placed in various social groups and observed for any color change. Observations indicated that the color shift can occur in as little as 10 seconds and appears to be affected by the sex and/or size of its companion cleaner. This was initially demonstrated by five cleaners that were caught while in juvenile coloration. They continued to display the juvenile pattern when placed in a tank with a larger female cleaner. When placed alone, with a larger male cleaner, or with other juvenile cleaners, they displayed the adult pattern. Interactions between small cleaners in juvenile coloration and adult females are rarely seen in the field (none were on Reef #40) but they apparently have an affect on the coloration shown by the small cleaner.

Cleaners have been seen to set up stations in the field and interact with a male while they are still small and in the juvenile color pattern (personal observation). This indicates that entering a social group is not the stimulus (or not the only stimulus) necessary for the color change. The purpose of this chapter's experiments is to find out exactly what stimulates the color change. The time frame and proximate reason for the coloration

change are determined using lab experiments, and then confirmed using a field experiment.

Methods

Color Change Test

To determine the cause of this phenomenon, 20 *L. phithiophagus* between 2.0 and 4.1 cm TL were caught while showing juvenile coloration. They were caught at various offshore locations around Oahu using handnets. Each was kept alone in a 20-liter aquarium or bucket. No host fish or other types of food were provided. After four days, 13 of the small cleaners had shifted to adult coloration, most prominently characterized by the dorsal anterior area of the body turning yellow. Each small cleaner was then placed in a 20-liter aquarium with an adult female and the color pattern of the small cleaner was observed. As a control, five cleaners between 4.1 and 5.0 cm TL were caught while showing adult coloration. They were held alone in a tank for four days, then placed with a larger adult female, and observed for any color change.

The same experiment was performed on 17 small *Labroides dimidiatus* that were caught using handnets at Lizard Island on the Great Barrier Reef. The shift to the adult color pattern was marked by the posterior ventral area shifting from black to light blue or white. Eleven of the small cleaners showed juvenile coloration at the time of capture (2.5 - 4.5 cm TL) and 6 were in the adult coloration (4.7 - 5.5 cm TL).

To see if this phenomenon occurs outside the genus *Labroides*, the same type of experiment was performed on two other species of wrasse, *Thalassoma duperrey* and *Coris gaimard*. *T. duperrey* is an endemic Hawaiian fish that is very common on the reefs of Kaneohe Bay and many other Hawaiian reefs. The adult has a greenish blue body with a broad orange bar behind the head, whereas juveniles show a black stripe over a

white stripe in the middle of the body and white to brown above and below the stripes. *Coris gaimard* is found throughout the central and western Pacific and has a very dramatic shift from juvenile to adult coloration. The juvenile has a bright orange-red body with five large black rimmed white spots, while the adult displays a maroon to green body with numerous small bright blue spots and a bright yellow tail. Both species could be frequently observed in an intermediate coloration, especially *C. gaimard*, in which the shift to adult coloration starts at the tail and gradually moves anteriorly, with the nose being the last part to show the juvenile coloration. Both *T. duperrey* and *C. gaimard* feed mainly on small invertebrates (Randall, 1996). They were caught using hook and line or hand nets.

Small adult-colored *T. duperrey* or *C. gaimard* were placed in a 200-liter aquarium with a larger female and watched for any change in coloration. Assorted other fishes were also in the aquarium. There were 10 replicates used for *T. duperrey* (3.7-6.2 cm TL) and 2 replicates for *C. gaimard* (13.1-14.3 cm TL).

Adult Chase Test

To further clarify the reason for the color changes seen in the previous experiment, small *L. phthirophagus* (3.7 - 4.1 cm TL, initially showing the juvenile coloration) were caught and placed alone in a small tank. The next day they were placed in a 20-liter tank with an adult female (6.6 - 7.5 cm TL). The number of times the small cleaner was chased was recorded over five minutes, grouped by 15-second segments. The small cleaner was then removed and placed in a separate tank until it shifted to the adult coloration (2-4 days). The small cleaner was then placed back with the adult female for a second treatment. The number of chases during each 15-second segment was recorded until the small cleaner shifted back to juvenile coloration. To ensure that any change in aggression was not an artifact of familiarity or the adult being confined in a tank for several days, a

third treatment was run the day after the second treatment. The small cleaner (in adult coloration) was chased with a hand net until it shifted to juvenile coloration, and then was placed with the same adult female. The number of chases was recorded every 15 seconds for as long as the small cleaner had stayed in the adult coloration in the second treatment. The total number of chases was recorded for the designated time period and compared between trials. Different cleaners were used in each of 12 sets of treatments.

Reef Placement Test

To verify that the coloration shift flexibility seen in the previous experiments is also seen in the field, small cleaners showing juvenile coloration were released at different distances from an adult female's cleaning station. Pilot studies showed that if juvenile cleaners are released into an appropriate environment (a small cave with adequate number of host fish in the area) there is a high probability (75-80%) that they will remain there for at least 2 to 3 weeks.

Fifteen small *L. phthirophagus* (3.6 - 4.1 cm TL, initially showing the juvenile coloration) were released into Kaneohe Bay at either 1m (± 0.5 m, N=7) or 5m (± 0.5 m, N=8) from the edge of an adult female *L. phthirophagus*' cleaning station (different stations were used for each small cleaner). They were all released near a small cave in the coral. The small cleaners were visited once a day until they shifted to adult coloration. On the second day after release they were observed for 30 minutes to ensure the 1m group was visited by the adult female at least once and the 5m group was not visited by the adult female. The number of times the adult chased the small cleaner during the 30-minute observation was recorded.

Short-Term Study (continued from Chapter 2)

In addition to the six social groups observed in Chapter 2, observations were made on a small isolated reef in the Sampan Channel. This was added to document unusual behavior being displayed by a small cleaner at the Sampan Channel reef. The cleaner was observed to be shifting from adult to juvenile coloration and back again. This behavior was documented and will be discussed. The Sampan Channel reef site was 2.5-3 meters deep, and observations were made using scuba. Because the reef is very small and contained only one social group, no control could be done.

Results

Color Change Test

Of the 20 *L. phthiophagus* that initially showed juvenile coloration, 13 shifted to adult coloration when isolated for two to four days. These 13 were between 3.0 and 4.1 cm TL. The seven that did not change were less than 3.0 cm TL. When placed with an adult female, all 13 *L. phthiophagus* that had shifted to adult coloration while alone shifted back to juvenile coloration within five minutes, always after being chased by the adult female. The sizes of the cleaners that shifted to adult coloration differed significantly from those that stayed in the juvenile coloration [Mann-Whitney U Test, $U=91$, $P < 0.005$, $N(\text{no shift}) = 7$, $N(\text{shift}) = 13$]. The five cleaners that were over 4.1 cm TL and were in adult coloration when caught stayed in adult coloration while also being chased vigorously (See Table 7). Four of the seven cleaners that shifted coloration (3.4-3.9 cm TL) were placed back with the adult female seven days after the initial shift but did not shift to juvenile coloration even after being vigorously chased by the adult. This indicates that there may be a limit to the time period during which they can shift back to juvenile coloration.

At Lizard Island three of the *L. dimidiatus* caught while showing juvenile coloration shifted to the adult coloration when isolated for four days. When placed with an adult female, they shifted back to the juvenile color pattern within 5 minutes. These three cleaners were all 3.9 to 4.5 cm TL. All the *L. dimidiatus* that were caught in juvenile coloration and did not shift to adult coloration while alone were less than 3.9 cm TL. The size of the cleaners that shifted to adult coloration differed significantly from those that stayed in the juvenile coloration [Mann-Whitney U Test, U=24, P <0.01, N(no shift) = 8, N(shift) = 3]. None of the *L. dimidiatus* that were caught while showing the adult coloration shifted to the juvenile coloration (see Table 7).

Table 7. Number of fish in each size category (T.L.) that shifted coloration when isolated (treatment 1) and when placed with a larger female (treatment 2).

Labroides phthiophagus

Size	<3.0 cm	3.0-4.1 cm	>4.1 cm
Coloration when caught	juvenile	juvenile	adult
Shifted coloration	0	13	0
Did not shift	7	0	5

Labroides dimidiatus

Size	<3.9 cm	3.9-4.5 cm	>4.5 cm
Coloration when caught	juvenile	juvenile	adult
Shifted coloration	0	3	0
Did not shift	8	0	6

In the experiments with *T. duperrey* and *C. gaimard*, all 12 showed signs of beginning to shift back to juvenile coloration. The 10 *T. duperrey* ranged from 3.7 to 6.2 cm TL and took from 2 to 8 days to show partial juvenile coloration, which consisted of a black stripe with white below visible on the side. The two *C. gaimard* were 13.1 and 14.3 cm TL and took 14 and 21 days to begin the color shift, which was defined as a visible white nose spot. The number of chases was not quantified, but the larger wrasse periodically chased the smaller wrasse.

Adult Chase Test

There was a significant difference among all three treatments (Friedman's Test, $\chi^2 = 12.67$, $P = 0.0018$). There was a significant increase in the number of chases from the first treatment (juvenile coloration, mean = 10.57 chases/min.) to the second treatment (adult coloration, mean = 15.65 chases/min., Wilcoxon's Signed-Ranks Test, $T_s = 3$, $P = 0.0047$, $N = 12$). The third treatment (juvenile coloration, mean = 10.34 chases/min.) was not significantly different from the first treatment (Wilcoxon's Signed-Ranks Test, $T_s = 26$, $P = 0.3078$, $N = 12$) but was significantly different from the second treatment (Wilcoxon's Signed-Ranks Test, $T_s = 0$, $P = 0.0022$, $N = 12$). This indicates that the adult females chased the small cleaners more when they showed the adult coloration, and the number of chases was not affected by familiarity or the time spent in captivity.

Reef Placement Test

Twelve of the small cleaners remained in place until they changed to adult coloration (six at 1 m from the resident adult cleaner, six at 5 m). The small cleaners released 1 m from the adult took significantly longer to shift to adult coloration than those released 5 m from the adult female's cleaning station [1m mean = 18 days, 5m mean = 6.33 days,

Mann-Whitney U Test, $U=33$, $P = 0.01$, $N(1m) = 6$, $N(5m) = 6$]. During the 30-minute observation on the second day, the six small cleaners at 5 m were never chased (or visited) by the adult female. The small six cleaners at 1 m were chased an average of 16.1 times during the 30 minute observation (chases ranged from 2 to 34).

Short-term Study

The small cleaner at the Sampan Channel reef (approximately 4 cm. TL) displayed a unique behavior: it periodically shifted from juvenile coloration to adult coloration and back again (average time between shifts- 5.4 minutes). Accompanying the shift to adult coloration was a move from a small cave (typical juvenile habitat) to on top of the reef (typical adult habitat) where it would interact with the male and two adult female cleaners in the social group. After a few minutes the small cleaner would then move back to the cave and shift back to juvenile coloration. During each period in the adult coloration the small cleaner received an average of 1.4 chases from the adult females and 2.2 'pass and quivers' from the male. It appears qualitatively that the hosts cleaned while in each color phase agreed with those cleaned by others of each coloration (see Table 6), after accounting for the lack of some host species at that reef. The small cleaner continued this behavior for four days and then displayed adult coloration full time.

Discussion

Within a certain size range the small *Labroides* have the ability to quickly shift from juvenile to adult coloration and back again. In *L. phthirophagus* this ability appears to be available for use once the cleaner reaches 3 cm TL and is then lost (or not used) after 4.1 cm is reached (3.9 and 4.5 cm, respectively, for *L. dimidiatus*). There also appears to be

a time limit on the ability to change, as small *L. phthirophagus* that have stayed in adult coloration for longer than about 10 days do not appear to be able to change back.

The increased aggression directed by the adult *L. phthirophagus* toward the smaller conspecific in adult coloration indicates that there may be an advantage to remaining in juvenile coloration. Even though the chases by the larger female appear to cause no noticeable physical damage (personal observation, through a microscope) there is still stress and the threat of damage. There also appears to be some advantage to displaying the adult coloration (further explored in the next chapter) as all cleaners in the proper size range shifted to the adult coloration after receiving no aggression for a few days. The ability to shift back and forth quickly may allow the small cleaner to ‘test’ the adult coloration and, if excessive aggression is received, shift back to the juvenile coloration.

The other wrasses (*T. duperrey* and *C. gaimard*) are also able to shift back to juvenile coloration when with a larger female. The process, however, is much slower, and takes days to weeks for the first sign of juvenile coloration to appear, versus minutes for a total shift in the *Labroides* species. It appears that the same evolutionary mechanism is at work in these species, but there is apparently no need for the non-*Labroides* wrasses to be able to shift back quickly to avoid aggression. The fact that the main source of food for these species is not from cleaning (Randall, 1996) may explain the difference and will be explored in subsequent chapters.

The unusual behavior seen by the small cleaner at the Sampan Channel reef at first appears to contradict the other findings of this chapter but there may be an explanation that agrees with the other results. It is hard to say if this one fish’s behavior is an anomaly or if this behavior occurs with every cleaner but for such a short time that it has not been observed before. The small size and isolated situation of this reef is unusual for Hawaii and the resulting social group may cause some unusual response from the small cleaner. The number of chases from the adult females appeared to be small and not enough to

cause an instant shift back to juvenile coloration. The small cleaner may be ensuring that there are enough hosts on top of the reef to sustain her and she is doing the shift gradually. The more cryptic host species (in the cave) may pose more for juvenile-colored cleaners and the small cleaner is taking advantage of its color shift ability to maximize its food during the transition

Chapter 4

Host Preference and Hunger Motivation Tests

Introduction

Because *L. phthriophagus* depends entirely on its hosts for food (Youngbluth, 1968), the preference of hosts can have a very significant effect on the selection of coloration displayed by the cleaner. The fact that small cleaner wrasses change to adult coloration before reaching maturity suggests an advantage to showing adult coloration, aside from mating. Losey *et al.* (1995) showed that at least one host fish identifies cleaners visually, therefore coloration may be important in attracting hosts to obtain food. The importance of food to a juvenile animal is well known (i.e., Bond, 1979) and this may be the reason for the quick coloration-change ability seen in Chapter 3.

The small cleaner's need for food may conflict with their need to avoid attacks from conspecifics. The host fish with more mucus, which are preferred by cleaners (Gorlick, 1980), are found above the reef. These host fish are generally cleaned by adults and might be expected to prefer the familiar adult coloration. The host fish species found in caves generally have less mucus and are therefore of less food value than the more obvious species. Increased hunger could have a motivational effect and increase the tendency to shift to adult coloration, allowing the small cleaner greater access to the food resources exploited by adults.

The experiments in this chapter were conducted to see if host fish have a preference for a particular cleaner coloration and if hunger is a factor in the coloration change. Two common host fish are tested for their preference in cleaner coloration, and then small cleaners are tested to see how hunger affects their shift to adult coloration. The experiments in this chapter used *L. phthriophagus* as the cleaner.

Methods

Doughnut tank host preference tests

Preliminary experiments using the doughnut tank at Coconut Island (a 12,000-liter tank shaped like a doughnut with interior-viewing windows consisting of 8 panes of glass) were performed to see if host fish prefer to pose for a cleaner that is displaying one of the two colorations. The doughnut tank was filled with an assortment of common reef fish and corals to simulate a natural reef environment.

Two similarly sized cleaners were used, one showing the juvenile color pattern and the other showing the adult color pattern (juvenile coloration mean - 3.5 cm TL, adult coloration mean - 3.8 cm TL). They were enclosed in separate clear plastic bottles (with holes to allow water flow) and placed in adjacent quadrants of the doughnut tank. Four trials were run with each of the cleaners in each quadrant for one trial, with the quadrant sequence randomized. Observations were made every 15 seconds (alternating between the two cleaners) for 10 minutes in each trial and the number of fish in each cleaner's quadrant and number posing for each cleaner were recorded. After moving the cleaners between trials, 5 minutes was given for the fish to acclimate to the new setup for the next trial. Each pair of cleaners was observed for a total of 40 minutes and 160 observations (80 observations for each cleaner). This experiment was run 8 times using different cleaners each time. At the end of each run the number of fish seen in each observation (posing or in the quadrant) was added up for the juvenile and adult-colored cleaners and the numbers were statistically compared. The host fish assemblage in the doughnut tank remained fairly consistent during the runs and consisted of 5-6 small *Dascyllus albisella*, 4-6 *Chaetodon* species, 6-8 Scaridae species, 4-5 Acanthuridae species, 2-3 *Canthigaster jactator*, 4-8 *Thalassoma* species, and 2-3 *Gomphosus varius*. The variation in numbers

was due to unanticipated losses and replacements. This experiment had several problems, such as the quadrant preference shown by the small *D. albisella* (see results). Another problem was that some fish, unconcerned with the cleaners, were swimming around the tank and would periodically be included in the 'quadrant' count, adding noise to the data. These factors demanded a more precise experimental and led to the use of a long tank, with only 2 host fish (see below).

Long tank host preference tests

Based on the results from the doughnut tank tests, it was decided that a more controlled experiment was needed to test for host response to the juvenile and adult color patterns. Two similarly-sized cleaners were used, as in the last test, one showing the juvenile color pattern and the other showing the adult color pattern (juvenile coloration mean - 3.4 cm TL, adult coloration mean - 3.9 cm TL). They were enclosed in separate clear plastic bottles (with holes to allow water flow) and then placed at opposite ends of a 3.6 m long outdoor tank (approximately 1200 liter capacity) that contained two adult *Dascyllus albisella*, a common reef damselfish that showed distinct posing behavior during the doughnut tank test. The tank had coral rubble at the center to be used as a shelter. The *D. albisella* were given at least four days to acclimate to the tank. To ensure that the *D. albisella* were fully acclimated, a pre-trial criterion had to be met before starting the experiment: after placing the bottles containing the cleaners in the tank, the *D. albisella* were observed to see if they visited each end of the tank during a 10-minute period. If this did not happen the cleaners were removed and the *D. albisella* were given another two days to acclimate. Once the pre-trial criterion was met, one of the *D. albisella* was randomly chosen and the time that it spent posing at each bottle and time spent in each end of the tank was recorded. Six 10-minute observations were made with the bottles containing the juvenile and adult cleaners being switched between each trial to control for

any preference the *D. albisella* had for one side of the tank. After moving the cleaners between trials 5 minutes was given for the fish to acclimate to the new setup. Different *D. albisella* and *L. phthiropagus* were used for each of 10 replicates.

This experiment was repeated using *Zebrasoma flavescens* as the host instead of *Dascyllus albisella*. All other aspects of the experiment were identical. *Z. flavescens* was used because it regularly travels over large areas of the reef, in contrast to *D. albisella* that seldom travels beyond a small home range (personal observation). Various species of butterflyfish (*Chaetodon miliaris*, *C. auriga*, *C. fremblii*) also were tried for this experiment but they preferred to stay in a corner of the tank instead of at the rubble in the center, resulting in a failure to meet the pre-trial criteria. Problems were also encountered when the experiment was tried with some cryptic species (*Myripristis* and *Apogon*) that would not leave the rubble area to pose for the cleaners.

Two host fish were used in the long tank experiment because *D. albisella* is a very social animal (personal observation) and keeping one alone would probably modify its behavior. Experiments using the doughnut tank demonstrated this fact as all the small *D. albisella* were frequently seen posing together. Any more than two would make it difficult to follow the one focal animal. For consistency, all host fish used in the long tank were kept in pairs.

Hunger motivation test

This experiment was performed to see if the availability of food (host fish) affected how quickly a small cleaner shifted to adult coloration when kept separate from other conspecifics. The time it takes for a juvenile-colored cleaner supplied with host fish to shift to adult coloration (control) was compared to the time taken for starved cleaners to shift.

To determine the time it takes to shift when food is supplied, a small cleaner (3.0-4.1 cm TL) was placed in 1.8 meter long tank (approximately 600 liter capacity) that contained between 10 and 18 host fish commonly found on Hawaiian reefs. At least 3 host wrasses were used in each trial as they are preferred host fish (Gorlick, 1980). The host fish were fed frozen brine shrimp daily and observed to see that they were allowing the cleaner wrasse to clean them. Host fish kept in aquariums with cleaners often tire of the constant cleaning and start chasing the cleaner away (personal observation). The large number of host fish used and the short time period for this experiment prevented any problems of this nature. In addition some host fish contracted a disease which made them more tolerant of excessive cleaning (the cleaners did not appear to be affected by this disease). The number of days it took for the small cleaner to shift to adult coloration was recorded. This trial was run 8 times with different cleaners in each trial. Host fish were used for a maximum of two trials. The data from this control trial was compared to the results from the Color Change Test in Chapter 3 where 13 small cleaners (3.2-4.0 cm TL) initially showing juvenile coloration were each isolated in a 20 liter tank or bucket with no food. The time it took each of these cleaners to shift to adult coloration was recorded.

Results

Doughnut tank host preference tests

There was no statistically significant preference seen for a particular cleaner coloration, for either number of host fish seen posing (adult mean = 67.4 fish, juvenile mean = 52.7 fish, Wilcoxon's Signed-Ranks Test, $T_s = 10$, $P > 0.05$, $N = 8$) or times host fish were seen in the cleaner's quadrant (adult mean = 124.1 fish, juvenile mean = 103.5 fish, Wilcoxon's Signed-Ranks Test, $T_s = 14$, $P > 0.05$, $N = 8$). These results may have been somewhat obscured by the preference of the small *D. albisella* in the tank,

which made up a large percentage of the fish seen posing (37%), for posing in one of the quadrants regardless of which cleaner was in that quadrant. This positional preference was shown to be significant by comparing the *D. albisella* posing in the preferred quadrant with their posing in the other quadrant, regardless of cleaner color (Wilcoxon's Signed-Ranks Test, $T_s = 5$, $P = 0.0391$, $N = 8$).

Long tank host preference tests

The *D. albisella* preferred the adult-colored cleaner as demonstrated by the time spent posing (adult mean = 0.50 min./10 min. trial, juvenile mean = 0.15 min. /10 min. trial, Wilcoxon's Signed-Ranks Test, $T_s = 0$, $P < 0.01$, $N = 10$) and time spent in that end of the tank (adult mean = 2.69 min. /10 min. trial, juvenile mean = 1.60 min. /10 min. trial, Wilcoxon's Signed-Ranks Test, $T_s = 0$, $P < 0.01$, $N = 10$).

The *Z. flavescens* showed preference for the adult-colored cleaner in time spent posing (adult mean = 0.75 min. /10 min. trial, juvenile mean = 0.56 min. /10 min. trial, Wilcoxon's Signed-Ranks Test, $T_s = 8$, $P = 0.049$, $N = 10$) but showed no significant preference in time spent in that end of the tank (adult mean = 3.09 min. /10 min. trial, juvenile mean = 2.42 min. /10 min. trial, Wilcoxon's Signed-Ranks Test, $T_s = 12$, $P = 0.11$, $N = 10$).

Hunger motivation test

The small cleaners kept in the tank with host fish available took significantly longer to shift to adult coloration than the cleaners that were given no food (Wilcoxon's Two Sample Test, $U_s = 104$, $P < 0.001$, $N_1 = 9$, $N_2 = 13$). The data had no overlap, as the starved cleaners took 2-4 days to shift coloration and the cleaners with host fish took 5-9 days to shift.

Discussion

The hunger test appeared to have very clean results but there may be some confounding factors. The fed cleaners were kept in a large tank and the starved cleaners were in a small tank. This was necessitated by the setup of the experiment and available tank space. As a check, a small cleaner in juvenile coloration was placed alone in one of the large tanks used for this experiment and it changed to adult coloration in 3 days which suggests that tank size was not a significant factor in the results.

Another possible confounding factor may be that the social contact with host fish that was denied to the starved cleaners affects the coloration shift. This social contact was necessary in the tests with fed cleaners because of the experimental design and the fact that cleaners will not eat any artificial food. The adding of any other animal to the tank with the starved cleaners would confound the test and was not tried. Despite these factors, it is believed that the experimental result demonstrates a motivation that affects the cleaner's color shifting behavior in the wild.

The long tank experiment showed that some hosts prefer to pose for adult-colored cleaners, indicating an advantage for a small cleaner to display adult coloration. The hunger motivation test showed that lack of food caused a quicker shift to adult coloration. These two experiments both support the hypothesis that a small cleaner would benefit by shifting to adult coloration as soon as possible. A juvenile cleaner without a dependable food supply might gain a sufficient benefit from the hosts that it could attract in adult coloration to balance the costs of increased attacks from adult cleaners.

The possible preference cryptic host species have for juvenile-colored cleaners does not contradict the results from this chapter. The cryptic species generally do not have the thick mucus layer preferred by cleaners (Gorlick, 1980), and a small cleaner would have to a station above the reef to access these preferred species.

Chapter 5

Final Discussion

The basic social structure for *L. phthirophagus* does not differ greatly from *L. dimidiatus* or from a general labrid model (Thresher, 1984). The *Labroides* species examined to date show a more structured home range than other labrids, but the nature of setting up a cleaning station may account for this difference. The differences between *L. dimidiatus* and *L. phthirophagus* may be explained by the fact that *L. phthirophagus* is more dependent on host fish for its food supply and would require that the cleaning stations be more separated to allow a higher density of host fish per station. The lower amount of movement demonstrated by *L. phthirophagus* may be explained by the need to spend more time at the station to ensure a reliable supply of host fish. Host fish are more likely to return repeatedly to a cleaning station that is occupied by a cleaner a high percentage of the time. Sex change by female *L. phthirophagus* apparently has a higher threshold, in terms of time between visits from the male, than in *L. dimidiatus*. This allows the male more time to establish a reliable group of host fish at his cleaning station. If female *L. phthirophagus* changed sex as quickly as female *L. dimidiatus* there would be an excessive number of males, and the increased mating opportunities that are currently enjoyed by the males would disappear. Selection pressure would then favor females who waited longer to initiate the sex change, and result in the current system.

The results from the *Labroides* aggression test are partly consistent with Fricke's (1980) theory that juvenile coloration masks the species identity, but the adult *L. phthirophagus* treated the juvenile-colored *L. phthirophagus* differently from any other fish (chasing or displays instead of cleaning). This indicates that there is recognition by the adult that the juvenile is of the same species, or at least different from all host species. The adult cleaners may have a specific image for the color pattern of fishes that are of the

most threat to them. This may be a 'search image' as used when animals are foraging (Lawrence, 1985 and Tinbergen, 1951) or a Sollwert 'reference value' (Hinde, 1966). The greatest threat is from fish that are conspecifics of approximately the same size or slightly smaller. These individuals are the most likely competitors for mates, food and dominance that control the opportunity to change sex. The decision rule is: anything that fits this 'reference value' and is of the same size or smaller is chased. The juveniles may be small enough to not be a significant threat to the adult and, by adopting a different coloration, avoid the more intense attack response evoked by the adult color pattern. Lack of competition prevents selection for a 'reference value' that leads to attack of juveniles. This allows them to stay near an adult cleaning station with a reliable supply of host fish.

The fact that the *D. albisella* and *Z. flavescens* prefer cleaners in the adult color pattern indicates that there may be better feeding opportunities for the cleaner when in the adult coloration. Even though juveniles clean a different assemblage of host fish, the preferred fish are those with the larger amount of mucus (Gorlick, 1980), and which are cleaned predominantly at adult cleaning stations. Host fish probably prefer the cleaner fish coloration that they encounter most frequently (Losey, 1979), and adult cleaners are more common above the reef (personal observation), where the fast swimming host fish are found.

Another possible factor in the habitat of juvenile cleaners is that, when very small, the cleaner may not be able to swim against the current and surge experienced in exposed locations where adults are found. By staying in caves and crevices the small cleaner avoids the hazards from these large water movements and is still able to find a sufficient number of host fish by cleaning the more cryptic species. As the small cleaner grows it is better able to handle the surges or current and is physically capable of swimming in a more open area.

After a certain minimum size is reached, the feeding advantages in adult coloration probably outweigh the disadvantages of being chased by adult females. Potential damage from a slightly larger attacker is less than that from a much larger attacker. As any animal gets larger its food requirements increase which makes it more important for the cleaner to clean hosts with more mucus. The ability to shift back and forth for some time period enables the cleaner to take advantage of the increased host availability when in the adult coloration while maintaining the option of cloaking themselves in juvenile coloration when the aggression received becomes excessive.

The fact that hunger is a motivating factor in the shift to adult coloration supports the rationale that the ability to access a broader supply of food is the reason for the early coloration shift (before maturity). This finding agrees with the results from the *D. albisella* and *Z. flavescens* preference test. An assessment is likely made of the energy reserves of the young cleaner and the amount of aggression received, and these are factored into the timing of the coloration shift, and possibly a change in cleaning station location. The level of aggression is monitored continuously and if it reaches some threshold, the coloration shifts back to the juvenile form. It is possible that once the small cleaner exhibits the adult coloration for a significant time period and does not receive excessive aggression, the chromatophores that enable the coloration change may be removed or made inoperable. This is most likely done to reduce the energy cost of maintaining these dynamic cells, along with the nervous or endocrine system needed to activate them.

The ability to quickly shift between adult and juvenile color patterns is uncommon and previously was noted in only one other species by Losey (1974b) who observed the color shift in *Aspidontus taeniatus*. This blenny not only mimics *L. dimidiatus* in adult and juvenile coloration, but it also has a cryptic coloration (mottled brown and silver-white) that it adopts when looking for a reef to settle on or moving between reefs.

Aspidontus taeniatus was generally thought to live by imitating cleaners and then biting unwary hosts (Randall *et. al.*, 1990), but Kuwamura (1983) showed that biting hosts was a very small part of its diet, which consisted mostly of demersal eggs and polychaete tentacles. The mimicking of the cleaner coloration appears to be mainly for predator avoidance. If there is already a relatively high density of *A. taeniatus* present on the reef then the resident *A. taeniatus* may chase away any new settlers. A newly settled *A. taeniatus* initially adopts the *L. dimidiatus* mimic coloration and, if it receives a large amount of aggression from conspecifics, it shifts back to the cryptic coloration, adopts a pelagic floating shape (curled) and drifts to another reef where there ideally would be a lower density of *A. taeniatus* (Losey, 1974). It appears to have a conflict similar to that in *Labroides* species where there is better feeding (or less predation) in the adult (mimic) coloration and less aggression in the juvenile (mottled) coloration, making it advantageous to be able to be able to shift between the two.

Even though many species have different juvenile coloration, the conflicting selection pressures to stay in juvenile coloration and to shift to adult coloration may not be strong at the same time. In many species the selection pressure to avoid aggression while small by displaying juvenile coloration is probably not countered until sexual maturity when the acquisition of territories or mates dictates a shift to adult coloration. For most species, coloration is not a significant factor in acquiring food. At maturity the animal is large enough that the danger from conspecific aggression is minimal, greatly reducing the advantage of being able to quickly shift back and forth in coloration. In fact many species show a gradual shift in coloration, as demonstrated by the occasional sighting of intermediate-colored individuals (*Thalassoma duperrey*, *Coris gaimard*, *Bodianus bilumulatus*, and *Pomacanthus imperator*, personal observation). These species may experience a sequential shift in strong selection pressures as opposed to the simultaneous selection pressures experienced by cleaners. In these cases with sequential selection

pressures a gradual coloration shift may be the most efficient method. *Thalassoma duperrey* and *C. gaimard*, the two examined species which show the gradual coloration shift, also showed the ability to reverse the shift when periodically chased by a larger conspecific. Since all the labrid species tested showed the ability to reverse coloration, it is logical to assume that most or all labrids have this ability. It is unclear whether juvenile fish in other families are able to reverse the coloration shift. An informative future experiment would be to test for this ability on a species with a different social structure, such as pomacentrids that guard individual territories.

The probable high cost of maintaining cells capable of quickly modifying their appearance is not worth the cost in these non-cleaner species, so a system of new chromatophore growth and old chromatophore death is probably used (Dickman et al., 1988). For these species the possibility of having to reverse the coloration shift is probably small so the more efficient one-way shift is used, and in rare instances the slow process is reversed. The need to stay in an area close to an existing adult social group, which is most likely important for cleaners, is probably not as critical in these other species. If excessive aggression is received the small fish can move to another suitable area without a major loss of feeding opportunities. This behavioral reaction avoids the possibly more costly physiological reaction of reversing the coloration shift.

The phenomenon of strong opposing selection pressures shaping the physiology of an organism could be found in other situations. The conflict between attracting a mate by displaying bright coloration and avoiding predation is a well known one (Endler 1980). Another commonly mentioned conflict is the one between foraging and avoiding predators. These are all strong selection pressures and the most common way of dealing with this conflict is to compromise. The animal would adopt a behavior or coloration midway between what would be optimal if only one selection pressure was present. One good example is presented by Endler (1980), who showed that female guppies prefer

males with large patches of bright colors that contrast with the background.

Unfortunately this would make the male conspicuous to a predation, so in areas with high predation pressure the males compromise by showing small patches of less bright colors. Even if the male guppy had the ability to perform a quick coloration shift it would not be advantageous, as predators would not give the guppy time to shift coloration before being preyed upon.

Most conflicts in selection pressure have predation as one of the pressures and the speed at which many predators act does not allow even a quickly modifiable physiological change that reduces predation risk. Most organisms are left with compromise as the most viable option. The conflicting pressures seen in cleaning wrasses may be fairly unique as each of the pressures will allow the cleaner time to assess the situation and, if necessary, perform a coloration shift without suffering irreversible effects.

As mentioned in the introduction, changing coloration is found throughout the animal kingdom, but the aquatic environment seems to be the primary location for rapid coloration changes, as many cephalopods and some crustaceans show very quick coloration changes (personal observation) in addition to the changes seen in fishes. The only obvious exception is in reptiles (Summers and Greenberg, 1995, Sherbrooke *et al.* 1994). The delayed plumage maturation seen in birds is temporally limited by the fact that they must wait for a molt to change coloration and cannot increase the rate of change to more than once or twice per season. Mammals have a similar problem as hair dominates the coloration and has to be shed and re-grown to change color. Quick coloration change requires an outer covering of living material in order for the change to be visible. Terrestrial arthropods could possibly perform the quick coloration change, but no references could be found.

The quick reversible coloration change ability seen here in *Labroides* species could be found in only in this specific circumstance, or it could be more common, but occur for

such a short time period that it is not easily seen. Now that this phenomenon has been examined, more research needs to be done looking specifically for conflicting selection pressures that may favor this ability. The extent of its occurrence is yet to be discovered.

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