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External tagging does not affect the feeding behavior of a coral reef fish, *Chaetodon vagabundus* (Pisces: Chaetodontidae)

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Synopsis

Increasingly, the ability to recognize individual fishes is important for studies of population dynamics, ecology, and behavior. Although a variety of methods exist, external tags remain one of the most widely applied because they are both effective and cost efficient. However, a key assumption is that neither the tagging procedure nor the presence of a tag negatively affects the individual. While this has been demonstrated for relatively coarse metrics such as growth and survival, few studies have examined the impact of tags and tagging on more subtle aspects of behavior. We tagged adult vagabond butterflyfish (*Chaetodon vagabundus*) occupying a 30-ha insular reef in Kimbe Bay, Papua New Guinea, using a commonly-utilized t-bar anchor tag. We quantified and compared feeding behavior (bite rate), which is sensitive to stress, of tagged and untagged individuals over four separate sampling periods spanning four months post-tagging. Bite rates did not differ between tagged and untagged individuals at each sampling period and, combined with additional anecdotal observations of normal pairing behavior and successful reproduction, suggest that tagging did not adversely affect individuals.

Introduction

Tagging to uniquely identify individuals has been a part of studying fish for well over a century (e.g., Atkins 1876). Various types of tags have led to major advances in our understanding of large- and small-scale movements, population dynamics, and behavior (Royer et al. 2005; McCormick & Meekan 2007; Verweij & Nagelkerken 2007; Wormald et al. 2008). Tags have been successfully employed in fish ranging from the largest (e.g., whale sharks, Wilson et al. 2007) to some of the smallest (e.g., pygmy gobies, Depczinky & Bellwood 2005). Tagging studies on coral reef fishes have a long history (e.g., Bardach 1958) and continue to provide a wealth of useful information.

Tagging technology has evolved to serve many purposes. Modern tags include simple pieces of plastic or other material externally attached to an individual (e.g., Rhodes & Tupper 2008); electronic transponders that record location, depth, water temperature, and a growing list of other parameters (Wilson et al. 2007); fluorescent elastomer gel tags injected subcutaneously (Bailey et al. 1998); and chemical markers incorporated into internal structures (Thorrold et al. 2006). An implicit assumption in studies that rely on data collected from tagged individuals is that these individuals are representative of the population; in other words, the tagging procedure and tag itself do not alter normal patterns of behavior. Although several studies have examined the effects of tags on relatively coarse metrics such as growth, survival and swimming performance (e.g., Moore et al. 1990; Adams et al. 1998; Ombredane et al. 1998), few have tested for effects on more subtle behavioral metrics such as feeding or reproduction (see review by Bridger & Booth 2003). In addition, the vast majority of studies investigating tagging effects focus on

relatively large, commercially important species, whereas most ecological and behavioral studies focus on smaller, unfished species.

Butterflyfish (Chaetodontidae) are conspicuous and colorful members of the coral reef fish community. Studies of this family have yielded a number of key insights in behavioral and reproductive ecology (e.g., Hourigan 1989; Tricas & Hiramoto 1992; Roberts & Ormond 1992). Butterflyfish appear to be time-constrained foragers that spend most daylight hours feeding (Gregson et al. 2008). Butterflyfish in distress tend to assume defensive postures and depart from their normal foraging activities (Yabuta 2000). As butterflyfish rarely utilize their maximum swimming abilities (Fulton 2007), we expect that the most significant sublethal effect would thus arise from a departure from normal feeding habits as opposed to effects on swimming ability. Furthermore, our study species (*Chaetodon vagabundus*) rarely demonstrates any aggressive or territorial behaviors (Berumen & Pratchett 2006), so it is unlikely that tagging would produce a noticeable change in this behavior. We therefore hypothesized that comparing the feeding behavior of tagged and untagged fish would be the most indicative and sensitive measure of whether external tags influence behavior.

Materials and Methods

We studied a population of the vagabond butterflyfish (*C. vagabundus*) occupying the 30-ha coral reef surrounding Kimbe Island (S 05° 12.112; E 150° 22.881) in Kimbe Bay, Papua New Guinea (see Almany et al. 2007) between December 2004 and April 2005. This species is widespread on Indo-Pacific coral reefs and reaches a maximum of approximately 21cm total

length and 150g (M Berumen, unpublished data). Butterflyfishes typically reach sexual maturity upon attaining adult size (Tricas & Hiramoto 1989), usually within 1-2 years (Berumen 2005). *Chaetodon vagabundus* adults form long-term, stable, monogamous pairs (Tanaka 1992). The goal of Almany et al. (2007) was to tag larvae produced by resident adults by labeling embryonic otoliths of larvae via maternal transmission of an enriched stable barium isotope (Thorrold et al. 2006). This larval tagging method requires capturing pre-spawning adults and injecting females with a small volume of isotope into the peritoneal cavity.

A team of four divers and snorkelers captured pairs of adult butterflyfish using a 2.5m x 1.2m monofilament barrier net with a 2-cm mesh size and hand nets. Because *C. vagabundus* is not sexually dimorphic, we injected each pair member with the isotope. We tagged each fish through the dorsal musculature ~5mm below the insertion of the dorsal fin with a 25mm-long external t-bar anchor tag (Floy® FD-94) using a tag applicator (Floy® Mark III). External tags prevented capturing and injecting the same fish twice, enabled us to make an accurate estimate of population size, and allowed us to test whether handling, isotope injection or external tags affected behavior.

We conducted observations of feeding behavior on both tagged and untagged individuals during four sampling periods: (1) prior to tagging (i.e., only untagged fish) in December 2004, (2) 5-8 days after tagging, (3) two months after tagging, and (4) four months after tagging. To avoid potential confounding effects, we limited observations to individuals that were part of a pair (Gregson et al. 2008). Pairs were selected haphazardly and the number of bites taken from the substrate by the focal individual during a single 3-min observation period was recorded by an

observer on snorkel that maintained an approximate distance of 2-4m from the focal fish (following Berumen et al. 2005). We only observed one individual from each pair. Feeding observations were primarily conducted in the afternoon or in the morning, but bite rate for *C. vagabundus* does not vary throughout the day (Gregson et al. 2008). We made the common assumption that bite rate is a valid metric for comparing feeding rates between tagged and untagged individuals as there is no reason to expect that tags would alter prey selection, availability, or quality for tagged fish relative to untagged fish. Further detail of prey choice and consumption would require observations at close distance that would disrupt normal behavior (see Berumen et al. 2005) or would require analysis of gut contents. Finally, care was taken to insure that a pair was only observed once during a given sampling period (by moving around the island in a clockwise fashion), but the possibility remains that some pairs were re-observed in different periods. Mean bite rates were compared using univariate ANOVA. All statistical analyses were conducted using SPSS® v16.0.

Results and Discussion

A total of 148 individual fish (53 tagged and 95 untagged) were observed across the four sampling periods, and the number of fish observed in each period varied (Fig. 1). This represents a subsample of the total number of fish tagged in Almany et al. (2007), as time did not allow for observations of every tagged individual. Bite rates ranged from 8 to 28 bites in a three-minute observation period for tagged fish, while bite rates ranged from 8 to 31 bites in a three-minute observation period for untagged fish. There was no evidence that mean bite rate differed among tagged and untagged individuals (ANOVA, $df = 6/147$, $F = 0.697$, $P = 0.653$), suggesting that tagging does not alter behavior. While previous studies have tested for effects of tagging on

survival, growth, and swimming performance (Bridger & Booth 2003), here we demonstrate that a commonly-utilized t-bar anchor tag has little influence on feeding behavior, a metric that is sensitive to stress in butterflyfishes (Yabuta 2000). Although we did not make attempts to quantify tag loss, we were able to find tagged fish several months after the initial tagging period. Techniques and technologies for tracking individual fish are constantly evolving, including genetic-based studies that do not require any external tags (Planes et al. 2009). In some cases, particularly with mobile fishes or over longer time periods, it is useful to have visual identification of tagged individuals, such as the T-bar anchor tags employed in this study.

The bite rates we observed in this study are comparable to those reported for *C. vagabundus* at other sites. For example, at Lizard Island on the northern Great Barrier Reef, Gregson et al. (2008) found that *C. vagabundus* had an overall mean bite rate of 6.41 bites per minute, only slightly higher than the overall mean bite rate of 5.91 bites per minute we found in the present study for untagged fish. This is similar to bite rates for other non-corallivorous butterflyfishes at Lizard Island (e.g., *Chaetodon ephippium*, 7.56 bites per minute; *Chaetodon semeion*, 5.97 bites per minute) (Gregson et al. 2008). More importantly, bite rates in the present study did not vary between tagged and untagged fish. In addition, we made several anecdotal observations during the course of the study lend additional support to the conclusion that tagging had little impact on behavior. First, we made feeding observations on four individuals within one hour of tagging and found bite rates similar to untagged fish. Second, when released by divers after tagging, adults quickly re-formed pairs and did not display any atypical avoidance behavior of divers or snorkelers. Third, tagged individuals successfully reproduced in the weeks following tagging (see Almany et al. 2007). These lines of evidence suggest that tags and tagging had little impact

143 on behavior and reproduction, and that the assumption that tagged individuals are representative
144 of the larger population is, in this case, valid.

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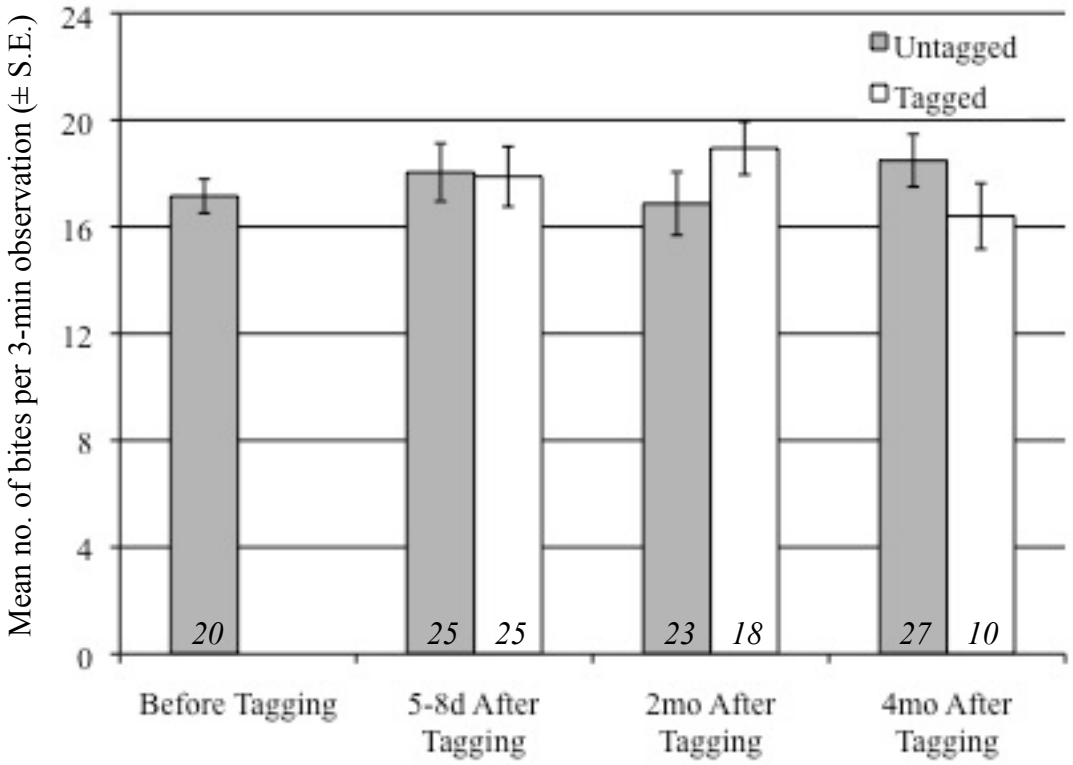
Figure 1. Bite rates of untagged (grey bars) and tagged (white bars) *Chaetodon vagabundus* from Kimbe Island, Papua New Guinea, prior to and at three periods following tagging. Bars indicate mean bite rate during 3-min. observations (± S.E.). Italicized number inside each bar indicates sample size. Means did not vary significantly from each other (ANOVA, df = 6/147, F = 0.697, P = 0.653).

224 Figure 1.


Mean no. of bites per 3-min observation (± S.E.)
24
20
16
12
8
4
0
Untagged
Tagged
20
25
25
23
18
27
10
Before Tagging
5-8d After Tagging
2mo After Tagging
4mo After Tagging