

A quantitative analysis of ferric iron in butterflyfish teeth (Chaetodontidae, Perciformes) and the relationship to feeding ecology

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Despite much work on the iron compounds found on invertebrate and vertebrate teeth, no interspecific quantitative analyses of iron compounds in fish teeth have ever been conducted, nor in any vertebrate has such a study related the iron concentrations to the feeding ecology. This study reveals that ferric iron exists on the tooth cap of eight species of chaetodontid butterflyfishes. The iron layer appears early in tooth development and is darkest, and presumably more concentrated, towards the tooth tip. There are significant differences among the eight species, with those that feed on harder prey having more iron than those that feed on softer-bodied prey. Furthermore, there exist differences among the species that correlate with the degree of morphological specialization for feeding on hard-bodied prey. The iron layer, which has been shown by others to be harder than the underlying enameloid, probably represents a specialization to harden the teeth, resisting abrasion and cracking.

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En dépit de l'existence de nombreux travaux sur les composés du fer dans les dents d'invertébrés et de vertébrés, il n'existe pas d'analyses quantitatives interspécifiques des composés du fer dans les dents de poissons et aucune étude ne relie les concentrations de fer dans les dents de vertébrés à l'écologie de l'alimentation. Cette étude met en évidence l'existence de fer ferrique dans la couronne de huit espèces de papillons de mer. La couche de fer apparaît tôt au cours du développement de la dent et est plus foncée, donc probablement plus concentrée, vers l'extrémité de la dent. Il y a des différences significatives chez les huit espèces et les espèces qui mangent les proies les plus dures ont plus de fer que celles qui se nourrissent de proies à corps plus mous. De plus, d'une espèce à l'autre il existe des différences qui sont reliées à l'importance de la spécialisation morphologique rattachée à la consommation de proies à corps dur. La couche de fer, reconnue comme plus dure que la couche émaillée sous-jacente, représente probablement une spécialisation dont l'effet est de durcir les dents, les rendant résistantes à l'abrasion et au bris.

[Traduit par la revue]

Introduction

Studies on the structure of teeth and dentitions have documented differences in their shape, size, arrangements, and molecular and crystalline composition. An early finding was the presence of iron compounds on the teeth of invertebrates and vertebrates. Among these studies, Lowenstam (1962*a*, 1967) and Towe and Lowenstam (1967) reported iron in the form of magnetite in the radular teeth of chitons: Lowenstam (1962*b*) reported goethite in the radular teeth of certain marine gastropods; and Runham and Thornton (1967), Runham et al. (1969), and Mann et al. (1986) reported iron in the teeth of limpets. Kerr (1960) described tinted tooth tip enamel in the axolotl, which he believed to be due to haematite. Other pigmented and unpigmented teeth capped by an iron-rich coat have been reported in fishes, amphibians, rodents, and shrews by Schmidt (1958, 1969, 1971), Miles (1963), Randall (1965), Garant (1970), Shellis and Berkovitz (1976), Halse (1972*a*, 1972*b*, 1973, 1974), Halse and Selvig (1973), Dotsch and Koenigswald (1978), and Vogel (1984). Halse (1972*a*) reviewed earlier studies on iron in teeth.

Teleost fish teeth, composed of a form of dentine partly covered by an enameloid substance (Orvig 1977; Poole 1971; Shellis 1978), are often brown at the tips. Schmidt (1958, 1969, 1971) described the colored iron oxide that covers the distal part of the enameloid tip of many teleostean teeth. The iron pigmentation is restricted to the thin membrane on the surface of the tooth cap. The pigment is more intense at the tip, decreasing towards the base of the tooth. This iron pigmentation can be observed before the tooth erupts and is not caused by the effects

of certain foodstuffs, nor is it related to the iron content of the water (Schmidt 1971). Shellis and Berkovitz (1976) found that the enameloid and basal dentine of piranha teeth are covered by a thin cuticle which contains ferric iron. Scanning electron microscopy revealed the cuticle to be firmly bonded to the underlying enameloid. However, Halse (1972*a*) reiterated earlier studies that found that the iron-rich layer is an outer zone of the enamel in rat incisors.

Lowenstam (1962*a*) and Towe and Lowenstam (1967) showed that the magnetite in chiton teeth hardens the teeth. Because of their hard tooth caps, chitons and limpets are major excavating agents of the intertidal notches of tropical limestone islands (Lowenstam 1974). Runham and Thornton (1967) and Runham et al. (1969) found that the iron-rich areas of limpet teeth are harder and more resistant to wear than the iron-poor regions. The teeth of certain patellacean marine gastropods are capped with goethite of Mohs hardness close to 5. They are able to erode limestone and rocks with mineral hardness close to that of their denticles (Lowenstam 1962*b*). Vogel (1984) found that the iron-containing enamel of shrew teeth is more resistant to abrasion than adjacent non iron containing enamel. The former prolongs the usefulness of the cutting edge. Shellis and Berkovitz (1976) believe that the iron-rich cuticle of teleost teeth serves the same function as that of the shark tooth cuticle proposed by Preuschoft et al. (1974); that is, the iron inhibits the initiation of cracks in the underlying layers.

Many studies have correlated structural modifications of the dentition with the diet of fishes. These have included a correlation of the development of the levator posterior pharyngeal muscle with the hardness of prey in labrid fishes (Yamaoka 1978), the hardness of prey with the degree of development of the pharyngeal musculature as a whole (Yamaoka 1978; Ono

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and Kaufman 1983), the robustness and strength of the jaw and pharyngeal teeth (Yamaoka 1978; Fryer and Isles 1972; Greenwood 1974; Liem 1973; Ono and Kaufman 1983; Motta 1985), and dentition changes with ontogenetic changes in diet (Sage and Selander 1975; Turner and Grosse 1980; Grudzien and Turner 1984). This work is the first to quantify and compare the amount of iron compounds interspecifically in any vertebrate dentition and relate this to the diet.

Methods

Specimens of the following butterflyfishes (Perciformes, Chaetodontidae) were collected by spear in Hawaiian waters during the summers of 1984 and 1985 and frozen for subsequent tooth removal: *Chaetodon ornatissimus*, *C. trifasciatus*, *C. multicinctus*, *C. quadrimaculatus*, *C. kleinii*, *C. unimaculatus*, *Forcipiger flavissimus*, and *F. longirostris*. Choice of the species was determined by the fact that a great deal is known about their worldwide diets and feeding behaviors, and their diets are interspecifically diverse. The tooth-bearing premaxilla and dentary bones were surgically removed and cleaned, then ultrasonically cleaned in distilled water until most of the soft tissue was removed. The jaws and teeth were then air dried. Initially the intact teeth were examined under a dissecting microscope and the extent of the discoloration compared and described. The teeth were then stained for 15 min in Perl's reagent (Prussian blue) at room temperature following the Hutchison technique outlined in Humason (1979). In this test ferric iron is dissolved by hydrochloric acid and then reacts with potassium ferrocyanide to form the Berlin blue precipitate, ferric ferrocyanide (Pearse 1961; Humason 1979). The teeth were again examined under the dissecting microscope. Following this, upper and lower jaws from five or more specimens of each species were similarly water cleaned and air dried. From each specimen 1 upper and 1 lower jaw tooth was removed such that 10 teeth from five specimens of each species were examined. I tried to avoid teeth that were not fully developed (see Motta 1984) and those that were unduly abraded at their tips, and did not use the principal biting teeth. Therefore, I selected the darkest teeth with unworn caps. These were generally at or near the labial margin of the jaw. Teeth were mounted on aluminium plugs with double sided Scotch tape and carbon coated. The teeth were examined under a International Scientific Instruments model 40 scanning electron microscope equipped with a Robinson backscatter electron detector, a secondary electron detector, and a lithium drifted silicon energy dispersive x-ray detector with a Princeton Gamma Tech System 4000 x-ray microanalysis system. The system was calibrated on a copper and aluminium standard. The specimens were placed at a working distance of 35 mm at 0° tilt, 18 kV, and were spot examined at magnifications ranging from $\times 1000$ to $\times 1500$. The $K\alpha$ peaks of aluminium, phosphorous, chlorine, potassium, calcium, titanium, manganese, iron, and cobalt were recorded after 100 s. To remove background interference from the phosphorous, calcium, and iron values, counts from neighbouring elements were averaged and subtracted from these values. For example, Al and Cl lie on either side of the P peak, so the mean count for the former two is subtracted from the latter to give an approximate background-free count for P. Similarly the mean values for K and Ti were subtracted from the Ca count, and the mean Mn and Co value from the Fe count. As these adjusted counts will vary with the takeoff angle of each specimen, and microstructure and orientation of the teeth cannot be equated, the counts cannot be compared directly. Instead the ratio of the elements is compared. In addition to the analysis of the tooth tips from the upper and lower jaw of each species, a similar count was taken on the tooth shaft of two or more upper and lower teeth from each species. The adjusted counts for Ca, P, and Fe from the tooth tip and shaft for 40 teeth of the eight species were then calculated as the ratios of P to Ca+P+Fe, and of Ca to Ca+P+Fe to see if the relative counts of Ca and P changed from tip to shaft. Following this, the ratio of Fe to Ca+P $\times 1000$ for the tooth tip was compared within and among species.

Statistical analyses

Initially a Wilcoxon matched-pairs signed ranks test was conducted

on the P to Ca+P+Fe and Ca to Ca+P+Fe ratios for the tooth tips versus shafts. A matched-pairs test was necessary because the same tooth was used for tip and shaft measurements in every case. Secondly, for the Fe to Ca+P ratios, a paired comparisons two-way ANOVA randomized block test was run on these paired teeth (Table 1) to see if there was a significant difference between upper and lower jaw teeth of each species; there was none ($\alpha = 0.05$). A paired comparisons test was again necessary because upper and lower jaw teeth from the same individual were often used. The test showed significant differences in iron concentration among the individual specimens ($\alpha = 0.05$) (Sokal and Rohlf 1981). Following that, a bivariate plot of the iron ratio in the upper and lower teeth of all paired specimens in all species was constructed to see if iron concentration in the upper tooth of a species is correlated with iron concentration in the corresponding lower tooth. The points cluster around a line of slope one, indicating that as a whole, iron concentration in the upper jaw teeth of a specimen is correlated with iron concentration in the lower jaw teeth of that same specimen. Therefore, this indicates that the upper and lower jaw iron ratios of each species cannot be lumped together to produce a sample size of 10, because they are correlated. Following this, two separate one-way ANOVAs were conducted on the upper and the lower jaw teeth. These *a priori* tests indicated that there were significant differences among the species in both cases ($\alpha = 0.01$). Then an *a posteriori* parametric unplanned comparison *T*, *T'*, GT2, and Tukey-Kramer tests were individually applied to the upper and lower jaws separately with an experimentwise error rate of 0.05. Because these are multiple comparison tests, this error rate is conservative (Sokal and Rohlf 1981). From these methods, the test producing the most sensitive experimentwise error rate of 0.05 was used; this approach is valid (Sokal and Rohlf 1981). Finally, because *C. ornatissimus*, *C. kleinii*, *C. trifasciatus*, and *C. multicinctus* mostly feed on hard prey and appear to have more iron on their teeth, while *F. longirostris*, *F. flavissimus*, and *C. quadrimaculatus* are primarily soft-prey feeders and appear to have less iron on their teeth as a whole, the variances for these two groups were compared and found to be significantly different by an F_{\max} test. Therefore, a Mann-Whitney *U*-test was applied to the two groups, first for the upper teeth and then for the lower teeth, to see if the groups had significantly different iron concentrations. Following Sokal and Rohlf (1981) values that are not significantly different are joined by a line in Fig. 1.

Results

Examination of the unstained teeth in all species revealed that the brown-red discoloration was confined to the tooth cap (see Motta 1984 for anatomy of butterflyfish teeth). In fact, the discoloration was darkest at the very tip of the tooth cap, fading towards the proximal end of the cap. The shafts appeared to have no discoloration. The coloration appeared essentially equal in intensity in similarly placed teeth from the upper and lower jaws in all species except *C. ornatissimus*. In that species, the lower jaw teeth appeared darker than those of the upper jaw. In addition, the teeth of this species appeared much darker than those of other species, being almost black at the very tip. In all species except *C. unimaculatus*, the larger more labial teeth appeared to be darker per unit area than the smaller more lingual ones; however, this might be an optical effect from the larger labial teeth. On the contrary, the smaller, more lingual teeth of *C. unimaculatus* appeared darker per unit area than the very large labial teeth, which showed marked abrasion. Even replacement teeth in the earliest stages of development (Motta 1984) show discoloration of the tooth caps in all species. In species such as *C. ornatissimus* with very long teeth, the very early replacement teeth have lighter colored caps than the later replacement stages, such that there is a gradation of increasing color intensity as the tooth develops. When stained with Perl reagent the caps of all species turned deep blue indicating the presence of ferric iron. The shafts did not stain blue.

TABLE 1. Ratios of iron to calcium plus phosphorous ($\times 1000$) for the tooth tips of eight species of butterflyfishes

	<i>C. ornaticus</i>		<i>C. kleinii</i>		<i>C. trifasciatus</i>		<i>C. multinctus</i>		<i>F. longirostus</i>		<i>F. flavissimus</i>		<i>C. quadrimaculatus</i>		<i>C. unimaculatus</i>	
	No.	Ratio	No.	Ratio	No.	Ratio	No.	Ratio	No.	Ratio	No.	Ratio	No.	Ratio	No.	Ratio
Upper jaw teeth	O14	35.6	K11	34.4	T12	31.7	Mu13	46.0	F16	29.7	Ff8	27.4	Q4	20.6	U16	8.8
	O16	41.5	K12	32.0	T18	25.8	Mu14	43.5	F15	28.4	Ff14	32.5	Q5	24.7	U17	20.0
	O18	35.8	K13	39.7	T19	37.5	Mu15	48.2	F19	29.6	Ff15	32.0	Q6	27.8	U26	10.4
	O24	61.4	K14	53.6	T20	55.5	Mu18	28.7	F20	27.0	Ff16	25.6	Q12	32.7	U27	14.9
	O25	47.5	K15	32.4	T21	43.0	Mu19	25.5	F21	24.7	Ff17	30.1	Q13	23.6	U28	13.6
Mean	44.4		38.42		38.7		38.4		27.9		29.5		25.9		13.5	
SD	10.7		9.0		11.4		10.5		2.1		3.0		4.6		4.4	
Lower jaw teeth	O16	54.6	K11	37.0	T11	22.3	Mu3	26.8	F16	30.8	Ff8	21.1	Q4	22.1	U16	5.2
	O18	64.1	K12	46.3	T18	37.8	Mu5	29.3	F15	32.7	Ff14	40.2	Q5	26.2	U17	10.9
	O23	36.1	K13	29.3	T19	32.8	Mu14	44.7	F19	38.1	Ff15	32.3	Q6	26.7	U27	16.4
	O24	55.8	K14	37.3	T20	31.9	Mu18	22.8	F20	28.6	Ff16	29.4	Q12	22.8	U29	22.0
	O25	42.2	K15	29.9	T21	41.1	Mu19	42.1	F21	33.5	Ff17	30.0	Q13	27.5	U30	13.0
Mean	50.6		35.96		33.2		33.1		32.7		30.6		25.1		13.5	
SD	11.3		6.9		7.1		9.7		3.5		6.8		2.4		6.3	
Overall mean	47.5		37.2		35.9		35.8		30.3		30.1		25.5		13.5	
Overall SD	10.9		7.69		9.4		9.9		3.8		5.0		3.5		5.1	

NOTE: Specimen number (No.) is given before each ratio. Means and standard deviations are given for the upper and lower ratios, as well as for the pooled upper and lower ratios.

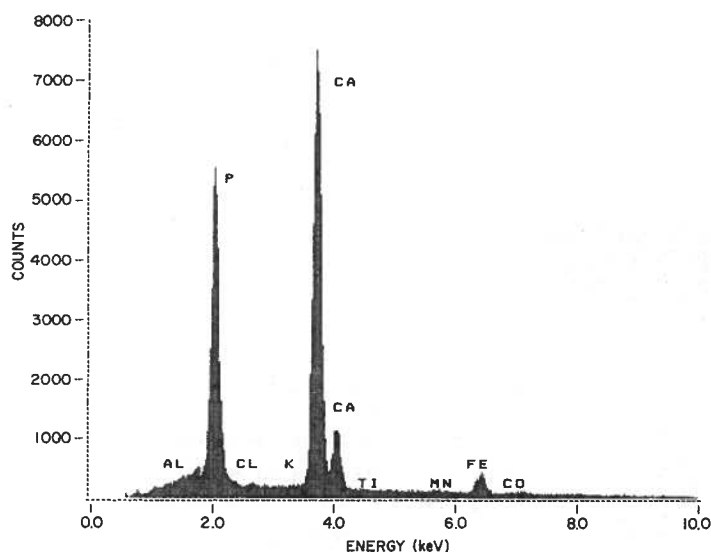


FIG. 1. Microprobe analysis of a representative tooth tip, *Chaetodon kleinii* specimen No. 5, lower tooth. Phosphorous, calcium, and iron peaks are shown along with the adjacent elements.

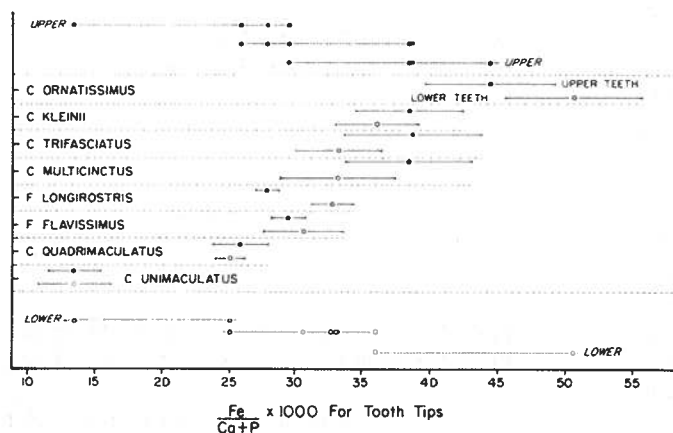


FIG. 2. Ratios of iron to calcium + phosphorous ($\times 1000$) for five upper and five lower tooth tips of eight species of butterflyfishes. Means and standard errors indicated. Solid circles, upper teeth; open circles, lower teeth. Values that are not significantly different at an experimentwise error rate of 0.05 are joined by lines at the top and bottom of the figure.

The x-ray microprobe analysis similarly revealed that iron was lacking on the tooth shaft. In some specimens, regions of the cap tip yielded little or no iron, yet adjacent spots yielded substantial iron counts. In such cases, iron counts were only collected in the region of higher iron concentration (Fig. 1).

The relative concentration of calcium did not change from shaft to tip ($\alpha = 0.05$); however, there was significantly more phosphorous on the tooth shaft than there was on the tip ($\alpha < 0.001$).

These data also suggest that there are statistically significant interspecific differences in the concentrations of iron on the tooth tips of the various species. Table 1 indicates the iron ratios for the tooth tips of the eight species. There were no significant differences in iron concentrations between upper and lower jaw tooth caps within any species ($\alpha = 0.05$).

Statistical analysis of the tooth iron concentrations revealed essentially three groupings ($\alpha = 0.05$, Fig. 2). In both upper and lower teeth there was a trend towards higher iron concentrations from *C. unimaculatus* to *C. ornaticissimus*. However, in the

upper teeth *C. ornaticissimus* was not significantly different from *C. kleinii*, *C. trifasciatus*, *C. multicinctus*, or *F. flavissimus*; it was greater than *F. longirostris*, *C. quadrimaculatus*, and *C. unimaculatus*, for example. The lower tooth analysis revealed that *C. ornaticissimus* and *C. kleinii* form a group with the highest iron concentration. The remaining species, including *C. kleinii* and excluding *C. unimaculatus*, form a second homogeneous group, and finally *C. quadrimaculatus* was not significantly different from *C. unimaculatus* ($\alpha = 0.05$).

Pooling the hard-prey eaters, *C. ornaticissimus*, *C. kleinii* (see Discussion), *C. trifasciatus*, and *C. multicinctus*, shows that the upper and lower tooth iron concentrations were greater than the upper and lower tooth iron concentrations of the soft-prey feeders, *F. longirostris*, *F. flavissimus*, and *C. quadrimaculatus* (upper $\alpha = 0.001$, lower $\alpha = 0.01$). In addition, the variance of the hard-prey feeders was significantly greater than that of the soft-prey feeders ($\alpha = 0.001$).

Discussion

The ferric iron described for these butterflyfishes matches the description for that of many other teleostean teeth (Schmidt 1958, 1969, 1971). The spotty distribution of iron on some tooth caps supports previous findings that the iron is deposited as a thin cuticle either overlying the enameloid (Shellis and Berkovitz 1976) or lying superficially within the enameloid layer (Schmidt 1969; Halse 1972a). Abrasion of the cuticle might account for the lack of iron in some regions. Halse and Selvig (1973) unexplainably found a spotty iron distribution in rat incisor enamel.

The iron cuticle in these butterflyfishes covers the tooth cap only, becoming more intense towards the tip. Similarly, even teeth that are apparently uncolored or only slightly colored red-brown, e.g., *C. unimaculatus*, have iron on the tooth cap. As in other teleosts, the coloration appears early in development before tooth eruption and apparently in the butterflyfishes it gets darker as the tooth develops. Garant (1970) found that during tooth development in *Helostoma temmincki*, a freshwater fish, cells of the inner epithelial layer of the tooth follicle accumulate large amounts of ferritin. The iron is subsequently deposited over the outer layer of the teeth, imparting a reddish brown coloration to them.

The reason for the decrease in the phosphorous ratio in the tooth tips compared with the shafts, unlike the unchanged calcium ratio, is not apparent. Halse (1972a, 1973, 1974) had somewhat similar findings in sectioned rodent teeth. In all but one of the species he examined, phosphorous and calcium concentrations decreased as iron increased on the tooth periphery. He concluded that the high occurrence of iron can hardly be explained unless the quantity of apatite is correspondingly reduced. Lemmings that had small iron concentrations unexplainably had no change in calcium and phosphorous across the tooth (Halse 1974).

The consensus is that the iron cuticle hardens the tooth cap and reduces abrasion and cracking (Lowenstam 1962a, 1962b, 1967; Towe and Lowenstam 1967; Runham and Thornton 1967; Runham et al. 1969; Lowenstam 1974; Shellis and Berkovitz 1976; Preuschoft et al. 1974; Vogel 1984), permitting the various organisms to feed on hard substrates.

If the butterflyfishes are grouped into those that feed on hard prey and those that feed on soft prey, the amount of iron on the tooth caps is significantly greater ($\alpha = 0.01$) for the hard-prey feeders (*C. ornaticissimus*, *C. kleinii*, *C. trifasciatus*, and *C. multicinctus*) than it is for the soft-prey feeders (*F. longirostris*,

TABLE 2. Summary of feeding habits of eight species of butterflyfishes under study

	Feeding habits	References
<i>Chaetodon ornatissimus</i>	Obligate hard-coral browser; scrapes with lower jaw	Hobson 1974; Reese 1977; Harmelin-Vivien and Bouchon-Navaro 1983; Motta 1985; P. J. Motta, manuscript in preparation
<i>Chaetodon kleinii</i>	Planktivore in Hawaii, omnivore in Okinawa, taking algae, octocoral polyps, anemones; takes coral polyps in southern Japan	Hobson 1974; Sano et al. 1984; Masuda et al. 1984
<i>Chaetodon trifasciatus</i>	Obligate hard-coral browser; scrapes and nips with jaws	Talbot 1965; Reese 1975, 1977; Neudecker 1977; Harmelin-Vivien 1979; Harmelin-Vivien and Bouchon-Navaro 1981, 1983; Anderson et al. 1981; Motta 1985; P. J. Motta, manuscript in preparation
<i>Chaetodon multinctus</i>	Obligate hard-coral browser; nips with jaws	Hobson 1974; Reese 1975, 1977; P. J. Motta, manuscript in preparation
<i>Forcipiger longirostris</i>	Suction feeder on small invertebrates, mostly shrimps	Hobson 1974; Anderson et al. 1981; Harmelin-Vivien and Bouchon-Navaro 1983; P. J. Motta, manuscript in preparation
<i>Forcipiger flavissimus</i>	Grasps and tears pieces of larger, benthic, noncoralline invertebrates	Hobson 1974; Anderson et al. 1981; Harmelin-Vivien and Bouchon-Navaro 1983; P. J. Motta, manuscript in preparation
<i>Chaetodon quadrimaculatus</i>	Omnivore that browses on algae, anthozoans, polychaetes, and hydroids	Hobson 1974; Reese 1975, 1977; Harmelin-Vivien and Bouchon-Navaro 1983; P. J. Motta, manuscript in preparation
<i>Chaetodon unimaculatus</i>	Facultative soft- and hard-coral grazer; lunges and bites	Hobson 1974; Reese 1975, 1977; Boucher 1979; Harmelin-Vivien 1979; Anderson et al. 1981; Tursch and Tursch 1982; Harmelin-Vivien and Bouchon-Navaro 1983; P. J. Motta, manuscript in preparation

F. flavissimus, and *C. quadrimaculatus*). *Chaetodon ornatissimus* is morphologically a very specialized hard coral browser that primarily utilizes its lower brush-like teeth to comb the hard coral surfaces (Motta 1985; P. J. Motta, manuscript in preparation; Table 2) (a coral browser removes soft tissue without damaging the calcareous skeleton, whereas a grazer takes skeletal material and soft tissue).

The feeding habits of *C. kleinii* are more varied. It feeds on algae, octocorals, and anemones in Okinawa (Sano et al. 1984; no mention is made whether algae is calcareous or noncalcareous), coral polyps in southern Japan (Masuda et al. 1984), and zooplankton in Hawaii (Hobson 1974). Planktivory may be a relatively recent event for this species, as it is believed to be for another planktivore, *C. miliaris* (Ralston 1981). My morphological and behavioral studies indicate that *C. kleinii* and *C. miliaris* are equipped for planktivory to a certain degree, but are not very specialized in this regard. I agree with Ralston (1981) that these species are recent entrants into the planktivore guild (Motta 1985; P. J. Motta, manuscript in preparation). *Chaetodon trifasciatus* and *C. multinctus* are both obligate, hard-coral browsers (Table 2). Morphologically, *F. longirostris* is a specialized suction-planktonic feeder with long jaws and a reduced dentition (Hobson 1974, P. J. Motta, manuscript in preparation). Superficially *F. flavissimus* looks like the former, but it is specialized for grasping and tearing soft benthic prey and it has a well-developed dentition of small teeth (Hobson 1974; P. J. Motta, manuscript in preparation). In terms of its dentition and overall jaw structure, *Chaetodon quadrimaculatus* is perhaps the most morphologically generalized species I

have examined from the Hawaiian Islands. Its omnivorous diet includes many soft-bodied prey as well as some coral polyps (Table 2).

The reason for the significantly greater variance in the iron counts for hard-prey feeders compared with the soft-prey feeders is not obvious. Greater wear on the teeth of the former could result in the probability of sampling worn and unworn areas on the teeth.

The comparison among the species (Fig. 1) reveals that although there are not discrete significant differences among most of the species, *C. ornatissimus* has darker teeth, and has higher iron readings and presumably more resistant teeth than many of the species, and *C. unimaculatus* has very lightly discolored teeth and significantly less iron than most of the species ($N = 5$, $\alpha = 0.05$). I believe that more extensive sampling would lead to clearer separation of the species, however. Halse (1972a) has also found a close correlation between the degree of pigmentation and iron concentration of rat incisor enamel. *Chaetodon unimaculatus* is the only coral-grazing butterflyfish in Hawaii (Hobson 1974; Reese 1975, 1977; Motta 1985). It has a robust jaw, with extremely robust, spatulate teeth. By lunging at both hard and soft corals it breaks off pieces of the corallite along with the soft tissue (Motta 1985). Its peripheral teeth show abrasion and appear lighter in coloration; therefore, I removed more lingual teeth for examination in this species. These teeth are not the principal biting teeth and, therefore, I believe the relatively low iron values are not due to abrasion. To corroborate the low iron counts on this species I tested numerous peripheral and more lingual teeth with

the same result. It appears that rather than having high iron concentrations on the tooth tips to reduce wear, this species has extremely robust teeth.

Higher relative iron counts in some of these fishes could be due to at least three nonmutually exclusive factors: (i) a relatively thicker iron cuticle; (ii) a cuticle of higher iron concentration per unit area; and (iii) a very low takeoff angle of the electron beam on the specimen. Although the takeoff angle could not be equated in any of the irregularly shaped specimens, I tried to control it by examining all teeth in the same manner. Furthermore, if this angle varied considerably among specimens I would expect greater variability in iron levels within a species, and less correlation between diet and iron counts. Halse's (1972a) close positive correlation between the degree of pigmentation and the iron concentration in rat incisor enamel suggests that I am most likely measuring iron concentration directly. Regardless of whether the iron cuticle varies in concentration or thickness among species, those teeth with relatively more iron will be more resistant to wear and cracking than those with less iron.

In conclusion, chaetodontid butterflyfishes are found to have ferric iron confined to the tooth cap. This cuticle appears during development and is darkest towards the tooth tip. Quantitative microprobe analysis reveals that there is no significant difference in iron content between upper and lower jaw teeth within species, but there are significant differences among species that appear to be related to their feeding ecology. Hard-coral browsers have a higher iron concentration than soft-prey feeders and, presumably, teeth that are more resistant to wear and cracking. There are trends that indicate that the specialized hard-coral browser, *C. ornaticornis*, has among the most resistant teeth. The hard-coral grazer *C. unimaculatus* has among the lowest iron concentrations, and instead has extremely robust teeth.

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