Barotrauma-associated regurgitation of food: implications for diet studies of Hawaiian pink snapper, *Pristipomoides filamentosus* (family Lutjanidae)

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Abstract.—Juvenile (128–244 mm fork length) pink snapper, *Pristipomoides filamentosus*, were caught by hook and line from 60–90 m depths offshore of Kaneohe Bay, windward Oahu, Hawaii, during February–August 1994. About one-half of the 180 specimens were intercepted by scuba divers 15–18 m below the sea surface and individually "bagged" live before they were retrieved for the remaining distance to the surface. The other half were retrieved directly by fishing line to the surface ("unbagged"); these latter fish thus remained at a continual risk of prey loss from regurgitation while they were stressed by the full extent of pressure change. The retained stomach contents of bagged and unbagged fish were compared on the basis of volume and type of food and on the size of individual prey items. Bagged samples of juvenile snapper on average retained a 116% (95% CI=70–157%) greater volume of prey than unbagged snapper; bagged snapper also had more types and greater maximum body sizes of prey than did unbagged fish. These results are discussed in terms of designing quantitative diet studies for juvenile snapper and other deep-water physoclistous fishes.

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The diet and feeding habits of deep-water physoclistous fishes are usually described by using fish collected with gears (hook and line, set lines, trawls, nets, traps) that subject specimens to the stress of large reductions in ambient pressure (barotrauma) as they are retrieved. Numerous studies have derived estimates of stomach fullness, prey consumption or daily ration, and gastric evacuation rates (reviewed by Bromley, 1994) based on collections that include or exclude specimens with empty stomachs. Often overlooked is the distinction between naturally occurring empty stomachs and stomachs that have partially or totally everted their contents as a result of swimbladder expansion during gear retrieval or other procedures of capture (Kohler and Fitzgerald, 1969; Daan, 1973; Durbin et al., 1983; Rice, 1988; Hislop et al., 1991).

The Hawaiian pink snapper or "opakapaka" (*Pristipomoides filamentosus*) represents a major portion of the deep-slope (150–300 m) bottomfishery in Hawaii (Haight et al., 1993a). Juveniles inhabit a shallower (60–90 m), relatively narrow depth zone on insular shelves (Parrish, 1989; Ellis and DeMartini, 1995; Moffitt and Parrish[1]) but, nonetheless, experience barotrauma during conventional collection procedures (Parrish and Moffitt, 1993). Juveniles are discontinuously distributed, and localized patches of occupied habitat have recently persisted for at least five consecutive years (1989–93; Parrish et al.[2]). One hypothesis for the persistence of these patches of juveniles is the localized distribution of prey, which in turn might reflect static substrate characteristics or recurrent patterns of near-bottom water movements. Testing such hypotheses requires describing the diet of juveniles as well as the distribution and availability of prey organisms.

[1] Moffitt, R. B., and F. A. Parrish. 1995. Habitat use and life history of juvenile Hawaiian pink snapper, *Pristipomoides filamentosus*. Honolulu Lab., Southwest Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, 2570 Dole St., Honolulu, HI 96822–2396. Unpubl. manuscript.

[2] Parrish, F. A., E. E. DeMartini, and D. M. Ellis. 1995. Effects of physiography and coastal discharge on the longshore distribution of juveniles of the snapper *Pristipomoides filamentosus* in the Hawaiian Archipelago. Honolulu Lab., Southwest Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, 2570 Dole St., Honolulu, HI 96822–2396. Unpubl. manuscript.

As part of our studies of the foraging and distribution of juvenile pink snapper, we have been developing methods to quantify stomach contents with acceptable accuracy and precision. In this paper we describe and evaluate a method of collecting juvenile snapper for diet analysis that minimizes the problem of subsurface regurgitation of food as specimens are retrieved from depth.

Methods

Fish collection

Juvenile snapper were caught by hook and line from near-bottom depths of 60–90 m, about 3–4 km offshore of Kaneohe Bay, windward Oahu, Hawaii, on nine dates during the period February–August 1994. All fish were captured between about 0900 and 1500 h. On each date, at 15–18 m below the sea surface, about one-half of all fish (randomly selected) were "bagged" by scuba divers (using NOAA NITROX II [64% N_2, 36% O_2] to extend diving periods approximately twofold at these depths). Divers observed fish—underwater visibilities consistently exceeded 30 m—as they were reeled up to 15–18 m and subsequently to the sea surface and noted whether specimens regurgitated food just before or during the bagging process; if they did regurgitate, these fish were left "unbagged" (see below). Median time for collection (1055 h) was the same for both bagged and unbagged fish. Bagged fish were sealed live in individually marked canvas bags before they were retrieved the remaining distance to the surface. The other half of the fish (unbagged) were brought directly to the surface by the fishing line. All fish were reeled in at a rate of about 30 m per minute (Haight et al., 1993b). Once aboard ship, unbagged fish were also packaged individually in marked canvas bags. Fish were stored on ice in a cooler until they were frozen intact in their marked bags at the NMFS Honolulu Laboratory, 2–8 h after capture.

Stomach content analyses

Each frozen fish was thawed, removed from its bag, and the bag everted in the laboratory. The external body surface of each specimen and the inside surface of the bag were rinsed into a black paraffin-bottom dissecting tray. Any prey fragments in the fluid were collected. Gill rakers, esophagus, and pharynx (including vomerine and jaw teeth) of the specimen were examined for prey. Ruptured swim bladders were noted, and extent of stomach eversion according to a stomach eversion index (EvI) was recorded (on a scale from 1 [not everted] to 2 [partially everted] to 3 [fully everted]). Fish were then measured (mm fork length [FL]). Lastly, the stomach was excised and its contents saved, along with any prey items that were free in the coelom as the result of stomach rupture. All prey (regurgitated and present in the stomach) were pooled. Prey were then blotted damp on a paper towel, and a displacement volume (0.01 mL) was measured. Stomachs and prey were fixed in 3.7% formaldehyde (sea water buffered) for 1–2 months, then preserved in 60% EtOH. The eviscerated wet weight (EW) of each snapper was recorded to the nearest gram.

Prey were examined under a dissecting microscope at 8–50× and classified (when possible) to the level of suborder or order. The length (longest axis) of each prey item was recorded to the nearest millimeter.

Identifiable prey were divided a priori into four major types (Bowen, 1983) on the basis of probable microhabitat: 1) crustaceans—mostly shrimps and stomatopods of indeterminate epibenthic or lower water column habitats; 2) benthos—primarily demersal octopods, echinoids, and microgastropods representing mobile epifauna; 3) nekton—actively swimming water column fishes and squids; and 4) jellies—weakly mobile, gelatinous salps and heteropods.

Statistics

A nonparametric bootstrapping procedure was used to evaluate mean prey volume in bagged versus unbagged fish because we were interested in quantifying the magnitude of possible differences in prey volume between the two treatments, even though sampling dates were too few to evaluate normality accurately. The variables used for bootstrapping (n=1,000 iterations; Manly, 1991) were 1) the differences between the date-specific mean prey volumes for bagged and unbagged fish. Because sampling design alone might have incompletely controlled for body size and date effects on prey volume, we further evaluated 2) the differences between predicted and observed prey volumes in unbagged snapper. Predicted values for unbagged fish were calculated on the basis of prey volumes measured for bagged fish of the same weight and date, because prey volumes were positively related to body weight for bagged snapper only (see Results section). Predicted values for bagged fish were derived from stepwise multiple regression of prey volume on sampling date and body weight. For both 1) and 2), date-means were weighted by the number of sample fish on that date.

Standard nonparametric tests (Siegel and Castellan, 1988; SAS, 1988: Proc NPAR1WAY, Proc FREQ)

were used to compare prey composition and prey size between bagged and unbagged fish. All tests were two-tailed (α=0.05). The Bonferroni correction (α'=0.05/m; Manly, 1991, p. 52) was used to control type-I error for $m = 3$, 2×2 chi-squared tests of presence or absence of prey types between treatments. Data were too few to analyze by separate chi-squared tests for each date. A three-way G-test was inappropriate for simultaneous comparisons of prey types because the occurrences of types were interdependent.

Results

Fish specimens

A total of 180 juvenile snapper (88 bagged, 92 unbagged) were collected on the nine sampling dates. From 5 to 40 specimens were collected per date (numbers of bagged and unbagged fish were <15% different on six of the last seven dates). Median size of fish was 176 mm FL (range 128–244 mm) and 98 g eviscerated weight (EW, range 36–289 g). No significant difference existed between bagged and unbagged fish, and growth was clearly evident over the sampling period (Table 1). Few bagged (n=3) and unbagged (n=3) snapper had ruptured swimbladders, and both bagged and unbagged fish had equivalently high indices of stomach eversion (Table 1). Four specimens had completely empty stomachs, and all were unbagged fish. Regurgitated prey were typically evident inside the bags of bagged fish and on gill rakers or elsewhere in the pharynx of both bagged and unbagged fish. Divers witnessed unbagged snapper frequently regurgitating stomach contents between 15–18 m depth and the sea surface, as the volume of gases in swimbladders expanded by more than an atmosphere of pressure, equivalent to 40% of all bladder expansion between depths of 75 and 15 m. Snapper were never seen regurgitating prey below 18 m, although safety considerations precluded prolonged observations by divers at depths >18 m.

Prey volume

From bagged snapper, we collected an average 108% more prey (0.75 ±0.57 mL [SD], versus 0.36 ±0.24 mL for unbagged fish), on the basis of the unadjusted grand means of all nine date-means weighted by their respective sample sizes (Fig. 1; Table 1). The logarithm of prey volume was positively related to the logarithm of fish body weight for bagged fish (P<0.0001) and additionally differed among collection dates (P=0.04; forward stepwise regression, P=0.15 as inclusion level; Proc REG: SAS, 1988; R^2=0.98). Date effects thus represented more than just temporal differences in fish size. A fish body weight effect on prey volume was undetectable (P>0.95) for unbagged fish, for which only collection date was important (R^2=0.93). A general date effect increased monotonically over the sampling period.

Prey volumes for both bagged and unbagged snapper were evaluated on the basis of bootstrapped estimates for the last seven sampling dates on which

Table 1

Summary statistics for juvenile pink snapper, *Pristipomoides filamentosus*, caught by hook and line off Kaneohe Bay, windward Oahu, Hawaii, during February–August 1994. Values for fork length (FL, mm), eviscerated body weight (EW, g), and the stomach eversion index (EvI) are medians for the sampling date; number of fish (n) is the date-specific sample size.

	Bagged					Unbagged				
Sampling date	n	FL	EW	Time caught	EvI	n	FL	EW	Time caught	EvI
09 Feb	1	155	57	0935	3	4	190	118	0920	2
28 Feb	4	168	87	1206	2.5	2	165	98	1006	2
02 Mar	16	150	60	1130	2	16	153	63	1143	2
04 Mar	14	155	64	1120	3	16	187	101	1104	2
15 Jul	9	172	87	1112	2	8	170	84	1105	2
19 Jul	11	173	94	1045	2	12	166	85	1045	2
11 Aug	19	198	138	1050	2	21	202	154	1044	2
16 Aug	6	187	116	1112	3	3	202	150	1025	2
18 Aug	9	184	105	1055	2	10	186	112	1055	2
All dates	88	177	100	1057	2	92	175	97	1050	2

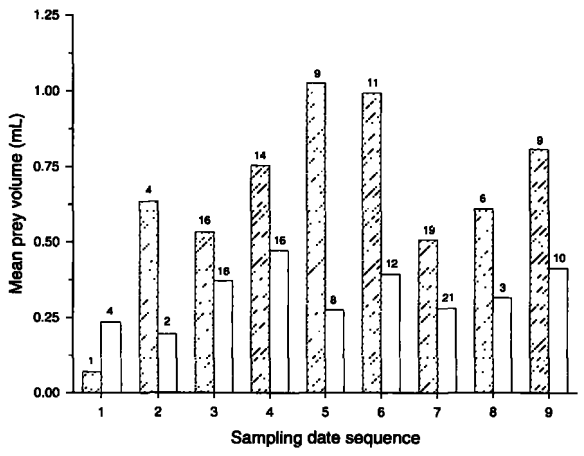

Figure 1

Mean prey volume for bagged (diagonal-line histograms) and unbagged (open histograms) juvenile pink snapper, *Pristipomoides filamentosus*, collected on each of nine dates during February–August 1994. Number of fish sampled is noted above histograms.

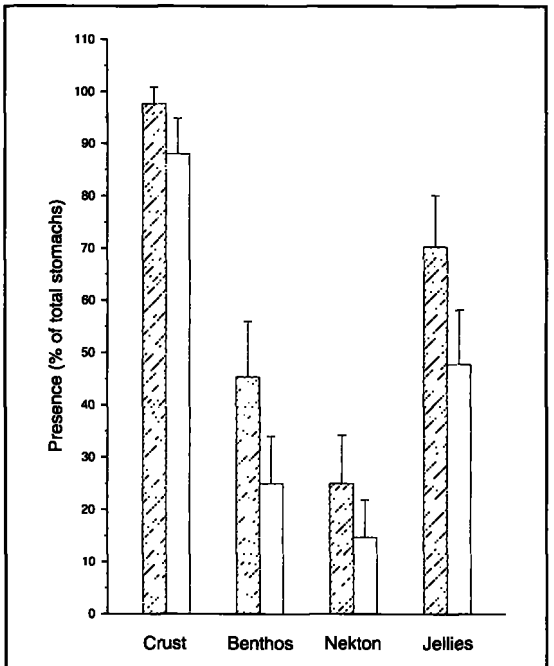

Figure 2

Percentage presence (1 standard error noted) for each of four major identifiable prey types (crustaceans, benthos, nekton, and jellies) for bagged (diagonal-line histograms) and unbagged (open histograms) juvenile pink snapper, *Pristimopoides filamentosus*, collected on nine dates during February–August 1994.

the smaller of the two sample sizes was nontrivial (≥3 fish, Table 1). Prey volumes bootstrapped without fish size and date adjustments averaged 112% (95% CI=72–152%) greater for bagged than for unbagged fish. For unbagged fish, predicted prey volumes (based on predicted values for bagged fish of the same body size and date) averaged 0.64 mL among dates, compared with observed prey volumes that averaged <0.30 mL. Bootstrapped differences of predicted minus observed prey volumes for unbagged snapper averaged +116% (95% CI=70–157%) or were 116% greater for bagged fish. Adjusted bootstrapped estimates thus were slightly larger than the bootstrapped estimates from unadjusted data.

Prey types

Three prey types (crustaceans, benthos, and jellies) were retained with greater relative frequency by bagged versus unbagged samples (2×2 Contingency χ^2 tests, $P<0.01$ for each of the three types; Fig. 2). A greater relative frequency of nekton for bagged (25%) versus unbagged (14%) snapper was only suggestive ($P=0.06$; Fig. 2). The proportion of fish with unidentifiable prey was similar in both bagged (49%) and unbagged (43%) fish (2×2 Contingency χ^2; $P=0.47$). Bagged fish in general retained greater numbers of major prey types (mean=2.9; median and mode=3) than did unbagged fish (mean=2.2; median and mode=2) (2-sample K-S test, $P<0.001$; Fig. 3).

Prey sizes

On average, the maximum size of prey retained by bagged samples was greater than the maximum prey size for unbagged samples (2-sample K-S test, $P<0.001$; Fig. 4). The minimum and median sizes of prey retained were equivalent, however, for bagged and unbagged (2-sample K-S tests, both $P>0.1$). Maximum prey size increased with fish body size for both bagged ($R^2=0.94$) and unbagged ($R^2=0.91$) snapper.

Discussion

Fundamental influences of ingestion

Two factors (fish body weight and date of collection) appear naturally to influence the amounts of prey consumed by juvenile snapper. The positive relation between fish size and prey volume was associated with snapper growth; juveniles averaged 50% greater body weight at the end versus the beginning of the 7-month period of study. Our observation of a monotonic temporal (date) effect in addition to the effect

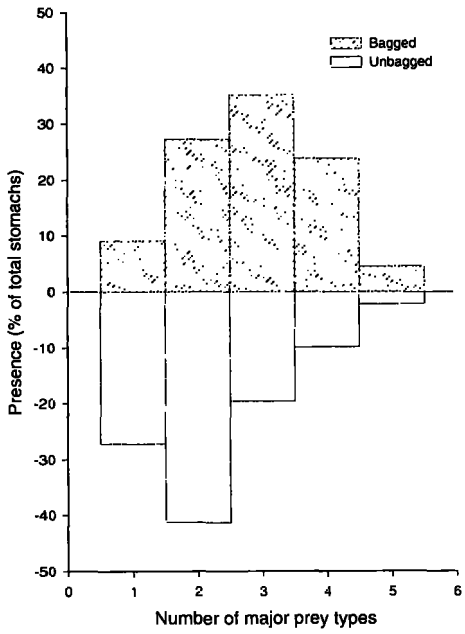

Figure 3

Frequency distributions for the major prey types (five, including unidentifiable category) present in bagged (above horizontal axis) and unbagged (below axis) juvenile pink snapper, *Pristimopoides filamentosus*, collected on nine dates during February–August 1994.

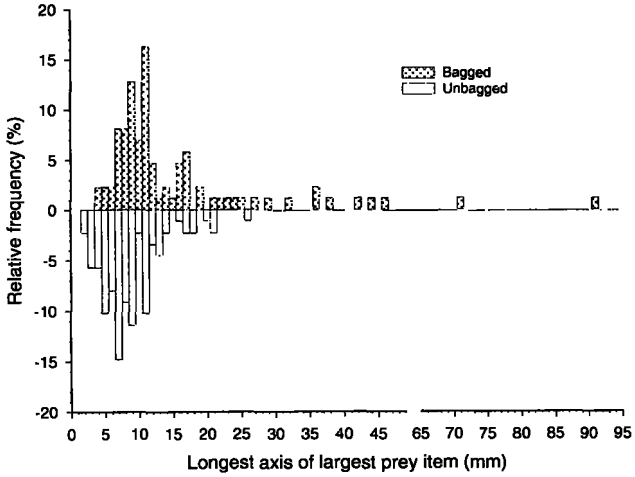

Figure 4

Frequency distributions of maximum prey sizes (all types, including unidentifiable category) for bagged and unbagged juvenile pink snapper, *Pristimopoides filamentosus*, collected on nine dates during February–August 1994.

of body size was not expected. It is possible that the date effect not related to size was caused by increases in the abundance or availability of prey between winter and summer.

In comparisons of prey volumes between bagged and unbagged specimens, the effects of body size and date of collection clearly need to be recognized, and adjustments made. Although this would seem intuitively necessary if sampling design does not control for fish body size and collection date, these factors influenced our results despite the fact that our sampling design specifically controlled for them. The possibility that the effects of body size and collection date may be too large to adjust statistically after-the-fact (if left uncontrolled by basic sampling design) should be taken into account in future studies.

Artifacts of regurgitation

Some food studies of physoclistous marine fishes (e.g. Bowman, 1986) have indicated or have strongly suggested that ingested prey are lost due to stomach eversion resulting from expansion of swimbladders when specimens are retrieved from even moderate (50 m) depths. This appears especially true for piscivores and other predators of large prey that have simple, undifferentiated stomachs (Treasurer, 1988).

Undetected regurgitation can lower the precision of estimates by adding erroneously empty or low-volume samples to the data set. There are also at least three ways in which undetected loss of stomach contents can bias dietary studies. These include 1) underestimates of the amount of food ingested, 2) underestimates of diet composition, including the taxonomic or functional variety of prey, and 3) size-selective regurgitation of prey. The latter can either inflate or reduce the effect of 1) depending on the nature of the size bias, which in turn may influence the precision of the estimates.

Precision of estimates

Total loss of stomach contents due to regurgitation occurred infrequently (4 out of 92 fish). However, the large reduction in prey volume for unbagged fish indicates that partial loss of prey because of regurgitation was a frequent occurrence. Partial prey loss did not inflate the variances of prey volume estimates for unbagged snapper; CV's (mean/SD·100%) of the date-specific estimates of prey volume were equivalent for bagged and unbagged fish (67% and 62%, respectively). Perhaps this reflects an effect of bias—selective loss of large prey—offsetting a decrease in

precision for unbagged fish. Species or collections in which the incidence of partial regurgitation is higher thus may be less precise, depending on whether loss of regurgitated prey is size-selective and in what manner so. High incidence of total regurgitation will be more likely to overwhelm size biases in retention and thereby lower precision.

Quantity and types of prey

Our observations for pink snapper document a general bias toward underestimating the total amount of food ingested by this species if partial or complete regurgitation goes undetected or is ignored. Because the number of different prey detected reflects the amount of material examined, underestimation of quantity should often underestimate prey variety as well, as our data illustrate. The observed underestimation for all four major prey types argues that our a priori classification of types, although subjective, was sufficient to detect a general bias resulting from regurgitation.

Sizes of prey

Size composition of prey can be biased by size-selective regurgitation. In the case of snapper, larger prey items were selectively regurgitated and lost by unbagged specimens. Large prey items might have been more recently ingested, despite likely crepuscular or nighttime periods of peak foraging (Haight et al., 1993b). The observed bias against retaining large prey may explain much of the underestimation of prey volume for unbagged snapper. Fishes with other feeding mechanism structures (e.g. different gill-raker size and spacing) may lack such a size bias, or regurgitation may result in the selective loss of smaller prey. Biases toward retaining larger prey can dampen the effect of regurgitation on underestimation of the total amount of prey.

Implications for diet studies

Depending on the specific research question, future studies of the food and feeding habits of juvenile pink snapper may benefit from the use of specimens collected at depth by divers. If simple comparisons of, say, the relative abundances of major prey types among fish collections are all that is needed, perhaps unbagged fish will suffice. Conversely, if longshore comparisons of large prey—perhaps comprising the bulk of the diet—are necessary, or if field estimates of total food intake or gastric evacuation rates are required, it is obvious that specimens retrieved directly to the sea surface will provide underestimates. Clearly, ingestion estimates for unbagged specimens will need to be adjusted for regurgitation loss, but collection date effects that are strongly nonlinear may make this difficult in practice. Further research is needed to develop methods for quantifying effectively the magnitude of regurgitation if collection date effects are nonmonotonic. Comparisons of diet among unbagged snapper captured at different depths will also be biased in terms of composition and size, as well as in terms of quantity of prey, because the amounts, diversity, and sizes of prey are interrelated and because the magnitude of regurgitation is influenced by extent of pressure change and hence depth of capture.

Bowman (1986) noted the progressive effects of depth on extent of regurgitation for other species. Prior explicit consideration of the interrelated biases resulting from regurgitation, on the composition, size, and quantity of prey has been lacking. The current consensus that diet descriptions require compound measures of both occurrence and bulk or mass data (Hyslop, 1980; Bowen, 1983; but see MacDonald and Green, 1983) reinforces the argument for critically evaluating prey data which may have been affected by barotrauma or other causes.

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