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Predator-induced changes in the diel feeding cycle of a planktonic copepod

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Abstract: An in situ manipulation experiment examined the effect of a diurnally foraging, zooplanktivorous fish, the three-spine stickleback *Gasterosteus aculeatus* Linnaeus, on the diel feeding cycle of the planktonic marine copepod *Acartia hudsonica* Pinhey. Copepods in the presence of fish had a significantly lower level of daytime gut fullness, and consequently a sharpened diel cycle of grazing, than did copepods in the absence of fish. The reduced level of copepod feeding could not be attributed to reduced availability of phytoplankton, such as has been previously suggested for vertically migrating animals residing at depth during the day. Rather, these changes in the feeding behavior of the copepods stemmed directly from the presence of predators. Due to reductions in motion and gut pigmentation, reduced daytime feeding may have adaptive value in reducing the copepods' risk of predation by making them less conspicuous to visual predators. Our results, combined with those of earlier studies, indicate that diel cycles of feeding and vertical migration can be separate, independent behaviors, although both likely have the same adaptive significance – predator evasion.

Key words: *Acartia hudsonica*; Diel feeding cycle; Diel vertical migration; *Gasterosteus aculeatus*; Predator evasion; Zooplankton

INTRODUCTION

Diel cycles of feeding in zooplankton, with higher levels of in situ gut fullness typically occurring at night, have been well documented in both freshwater and marine zooplankton (see review by Haney, 1988). Likewise, the occurrence of diel vertical migration in freshwater and marine zooplankton has been well documented (see reviews by Hutchinson, 1967; Longhurst, 1976; Raymont, 1983; Bayly, 1986), with daytime residence at depth most often attributed to avoidance of surface-dwelling, visually orienting predators (e.g., Zaret & Suffern, 1976; Gliwicz, 1986; Bollens & Frost, 1989a,b). However, there has been considerable debate as to whether these two diel cycles (migration and feeding) are separate, independent behaviors (e.g., Haney & Hall, 1975; Daro, 1985; Stearns, 1986; Haney, 1988; Dagg et al., 1989), or alternatively, that observed diel feeding cycles are simply the result of animals vertically migrating into and out of food-rich surface waters (e.g., Gauld, 1953; Haney & Hall, 1975; Daro, 1980; Simard et al., 1985).

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We report results of an in situ manipulation experiment examining the diel feeding cycle of the planktonic marine copepod *Acartia hudsonica* Pinhey in the presence and absence of a zooplanktivorous fish, the three-spine stickleback *Gasterosteus aculeatus* Linnaeus. An assessment of the vertical distributions and diel vertical migrations of *A. hudsonica* during this experiment is presented elsewhere (Bollens & Frost, 1991). In brief, Bollens & Frost (1991) found that adult female *A. hudsonica*, normally migratory (at the surface during the night, at depth during the day) in the presence of sticklebacks, would rapidly (12–24 h) switch to non-migratory behavior in the absence of sticklebacks. In the present study we asked two questions: (1) What effect does the presence or absence of visual predators have on the feeding cycle of *A. hudsonica?* (2) If such an effect is present, can it be distinguished from any changes in feeding that might occur as a result of diel vertical migration into and out of food patches?

Materials and Methods

The field site was Jakles Lagoon (48° 28′ N, 122° 59′ W, 2.6 ha, 3.9 m maximum depth), a marine lagoon on San Juan Island, Washington, USA. *A. hudsonica* and *G. aculeatus* are the overwhelming dominant mesozooplankter and zooplanktivorous fish, respectively, in Jakles Lagoon (Landry, 1978; Bollens & Frost, 1989b; Bollens et al., 1991), with invertebrate predators being extremely rare or absent (Landry, 1978; Bollens & Frost, 1989b).

We established eight 2600-l cylindrical (1 m diameter × 3.3 m depth) enclosures, constructed of 1.5 m wide panels of 10 mil (0.25 mm) woven polyethylene stitched together with double seams of natural fiber thread, in linear array along the south side of a raft moored in the center of the lagoon. These enclosures, having been soaked in saltwater for several weeks during previous years' experiments, were rinsed with freshwater, then refilled with filtered (73 μm) seawater pumped from $\approx$1.5 m depth, and allowed to stand for 36 h. On the night of 2 May 1990, populations of adult *A. hudsonica* were established in the enclosures at natural densities (9.6 ± 2.5 [$\bar{x} \pm$ SE] adult females $\cdot l^{-1}$) by emptying the contents of four vertical plankton hauls (0.5 m diameter net, 253 μm mesh, closed cod end), made from within 15 cm of the bottom to the surface, into each enclosure. Four replicates of each of two treatments were established: Fish (containing nine adult sticklebacks allowed to range freely throughout the bags) and No Fish. While the stocking density of sticklebacks used in this experiment was probably higher than occurs naturally in Jakles Lagoon in May, it was an order of magnitude lower than that for peak abundance of pelagic juveniles in August (Bollens et al., 1992).

Near noon and midnight on 7 May 1990 (5 days after the initiation of the experiment) we collected copepods from each enclosure by making a vertical tow with a 0.25 m diameter, 253-μm mesh plankton net fitted with a closed cod end. Copepods were immediately filtered via gentle aspiration onto a glass fiber filter which was then placed in a plastic Petri dish, wrapped in aluminum foil and frozen on dry ice; time from

collection to freezing was ≈3 min and never >5 min. Vertically stratified water samples were collected at three depth intervals (0.15–1.15, 1.15–2.15, 2.15–3.15 m) in each enclosure near noon with a multiple water bottle sampler described in Bollens & Frost (1989b); this method proved inadequate for collecting copepods in the Fish enclosures because copepods were too dilute ($<5 \cdot \text{sample}^{-1}$) to allow for analysis of gut pigments. Water samples were immediately frozen and the abundance of Chl *a* determined in the laboratory by fluorometric techniques (Strickland & Parsons, 1972).

Frozen copepod samples were examined under a dissecting microscope. For each sample, adult female copepods were individually rinsed in phytoplankton-free seawater and transferred to clean 25-mm Gelman type A/E glass fiber filters in subsample groups of 12–20 animals. The number of subsamples · field sample^{-1} ranged from 1 to 12 (4.0 ± 1.1 [$\bar{x} \pm$ SE] day subsamples, 5.9 ± 1.4 night subsamples), depending on the total number of copepods in the sample. Each subsample filter was then ground in an ice-bathed test tube containing 90% aqueous acetone and left in darkness for 24 h, with periodic shaking, to extract pigments. Each subsample was then centrifuged and Chl *a* and pheopigment *a* measured by fluorescence using a Turner Designs fluorometer. The general method was that of Yentsch & Menzel (1963) as described by Strickland & Parsons (1972), except that pigment was divided by number of copepods in the subsample rather than volume of water filtered. This fluorescence technique does not distinguish between pheophytin *a* and pheophorbide *a*; hence the term "pheopigment *a*" includes both compounds. Total gut pigment (Chl *a* + pheopigment *a*) · copepod^{-1} was determined for each subsample, and the mean of all subsamples calculated for each field sample, i.e., each replicate enclosure. In this manner we obtained four independent estimates of copepod gut fullness for each treatment (Fish and No Fish) and time (Day and Night).

A Model I two-way ANOVA was performed on these data after log transformation (Sokal & Rohlf, 1981) to test for the effects of treatment (Fish vs. No Fish) and time (Day vs. Night) on copepod gut fullness. A Model I two-way ANOVA was also performed on the water column Chl *a* data (log-transformed) to test for the effects of treatment (Fish vs. No Fish) and depth (upper, middle and lower water column) on abundance of water column Chl *a*.

Results

Abundance of gut pigment was significantly lower in copepods in the Fish treatment vs. the No Fish treatment (Fig. 1, treatment effect in Table I). Considering only the nighttime samples, mean abundance of gut pigment was 11% lower in the Fish treatment than in the No Fish treatment (Fig. 1), but this difference was not statistically significant (t test, 6 df, $p > 0.05$). However, mean daytime abundance of gut pigment was 61% lower in the Fish treatment than in the No Fish treatment (Fig. 1), a difference that was very highly statistically significant (t test, 6 df, $p < 0.001$).

A diel cycle of feeding was evident in the experiment as a whole (Fig. 1, time effect

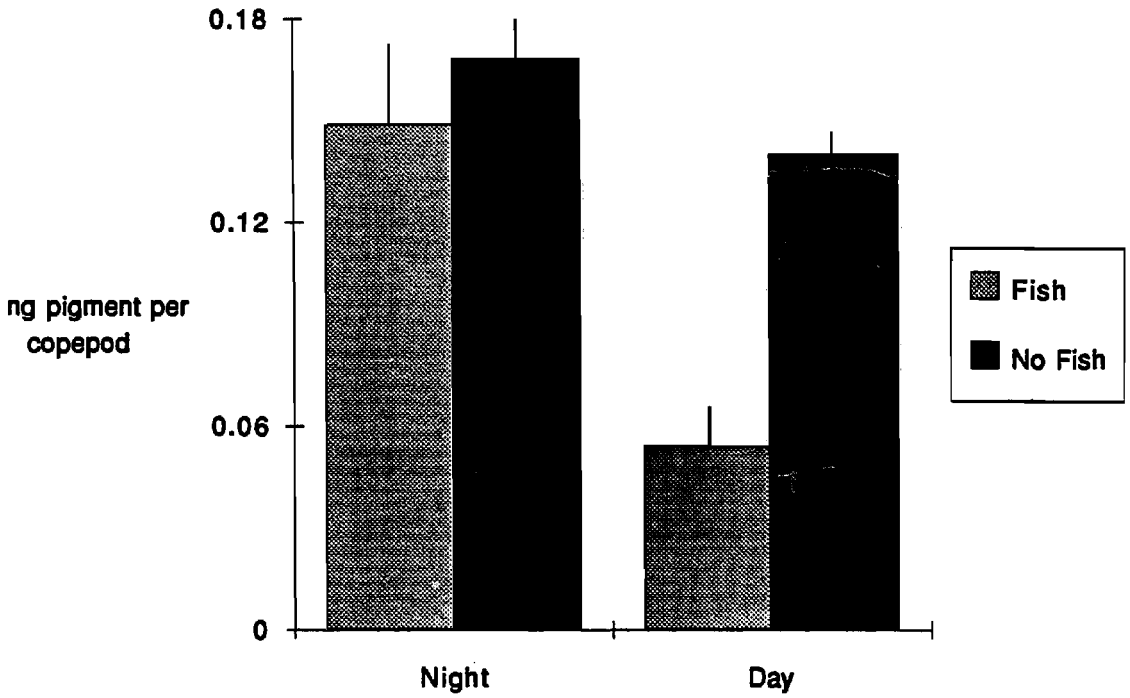

Fig. 1. Abundance of total gut pigment (Chl *a* and pheopigment) · adult female *A. hudsonica*$^{-1}$ in experimental enclosures, Jakles Lagoon, Washington, May 1990. Each column is $\bar{x} \pm$ SE of four replicate enclosures.

TABLE I

Two-way ANOVA table summarizing effects of treatment (Fish vs. No Fish) and time (Day vs. Night) on abundance (log-transformed) of gut pigment · adult female *A. hudsonica*$^{-1}$ in experimental enclosures in Jakles Lagoon, Washington, May 1990. Asterisks indicate level of significance: *** probability that observed *F* value occurred by chance is <0.001; ** probability that observed *F* value occurred by chance is <0.01.

Source of variation	df	SS	MS	*F*
Treatment	1	0.251	0.251	20.820***
Time	1	0.284	0.284	23.520***
Treatment × time	1	0.141	0.141	11.664**
Error	12	0.145	0.012	
Total	15	0.820		

in Table I), as well as in both treatments considered separately, with mean nighttime abundances of gut pigment being statistically significantly greater than mean daytime abundances (*t* tests, 6 df, $p < 0.05$). However, the magnitude of this diel difference was substantially larger in the Fish treatment, where daytime gut fullness was 64% lower than nighttime gut fullness, than in the No Fish treatment, where daytime gut fullness was only 17% lower than nighttime gut fullness (Fig. 1).

Abundance of Chl *a* in the water (Fig. 2) was statistically significantly greater in the Fish treatment than in the No Fish treatment (treatment effect in Table II), but was

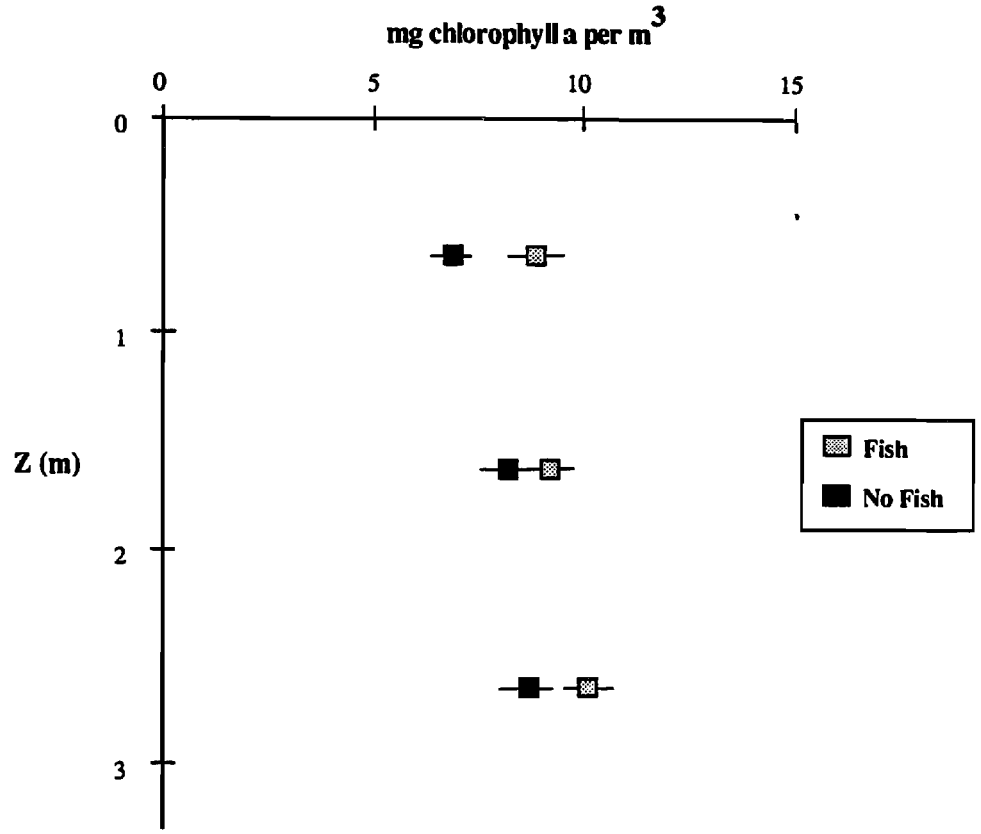

Fig. 2. Abundance of Chl *a* in experimental enclosures, Jakles Lagoon, Washington, May 1990. Each point is $\bar{x} \pm$ SE of four replicate enclosures and is located at mid-point of each depth stratum sampled.

TABLE II

Two-way ANOVA table summarizing effects of treatment (Fish vs. No Fish) and depth (upper, middle and lower water column) on abundance (log-transformed) of Chl *a* in experimental enclosures in Jakles Lagoon, Washington, May 1990. Asterisk indicates level of significance: * probability that observed *F* value occurred by chance is <0.05; all other *F* values are not significant (NS, $p > 0.05$).

Source of variation	df	SS	MS	*F*
Treatment	1	0.032	0.032	5.333*
Depth	2	0.022	0.011	1.833 NS
Treatment × depth	2	0.003	0.002	0.333 NS
Error	18	0.117	0.006	
Total	23	0.017		

vertically homogeneous, as evidenced by the absence of statistically significant differences among depth strata (depth effect in Table II).

DISCUSSION

The presence of three-spine sticklebacks, a predominantly diurnally foraging, zooplanktivorous fish (Wootton, 1984; Jakobsen & Johnsen, 1987), caused a reduction

in the level of daytime gut fullness (and by inference feeding) in adult female *A. hudsonica* in our experiment. This reduced level of daytime feeding acted to sharply enhance the diel cycle of feeding in the copepod. This reduced daytime feeding in the presence of fish could not have occurred as a result of overall lower phytoplankton abundance, as Chl *a* was actually more abundant in the Fish enclosures than in the No Fish enclosures (Fig. 2, Table II).

Reduced levels of daytime feeding by zooplankton in the field have often been attributed to greater vertical segregation of zooplankton and phytoplankton during the daytime, such as occurs when zooplankton undertake diel vertical migration in a vertically patchy food environment (e.g., Gauld, 1953; Haney & Hall, 1975; Daro, 1980; Simard et al., 1985). While we were unable to assess the vertical distributions of copepods concurrently with our sampling for gut fluorescence, Bollens & Frost (1991) determined the daytime distributions of *A. hudsonica* on several dates immediately preceding our sampling and found copepods in the presence of fish to consistently occur deeper in the water column than copepods in the absence of fish. Thus the copepods we sampled for gut pigments in the Fish enclosures were very likely deeper in the water column during the daytime than copepods in the No Fish enclosures. However, no significant differences in abundances of Chl *a* with depth were evident in our experiment (Table II). Thus the reduced level of daytime feeding of the copepods in the presence of fish could not have been the result of reduced food availability, either vertically or in total, but rather could only have been due to the presence of fish.

Stearns (1986) reported that adult female *Acartia tonsa* in the Newport River estuary, North Carolina, USA, showed approximately a 60% reduction in daytime feeding relative to nighttime feeding, and that copepods held for 60–70 h in the laboratory and placed in recently filtered seawater showed a significant negative correlation between grazing and light intensity. While these results clearly show that light can affect feeding in *A. tonsa*, these copepods had presumably experienced fish or fish-treated water either concurrently with the sampling (field study) or at least very recently (laboratory study). In the present study, adult female *A. hudsonica* showed a 64% reduction in daytime feeding relative to nighttime feeding in the presence of fish, but only a 17% reduction after copepods had been excluded from fish for 5 days. Collectively these two studies suggest the possibility of a hierarchy of environmental cues regulating daytime feeding in the copepods: under current or recent predatory threat, as sensed by the copepods via visual, mechanical or chemical signals emitted by fish, light cues act to trigger reduced daytime feeding in the copepods; in the absence of predatory threat and predator-mediated signals, light cues play little or no role in regulating copepod feeding.

A reduced level of feeding and gut pigment in the presence of visual predators could afford zooplankters an adaptive advantage in two ways. First, pigmentation is known to affect the susceptibility of zooplankton prey to visual predators generally (Zaret, 1972; Mellors, 1975; Kislalioglu & Gibson, 1976), and the occurrence of pigmented gut tubes in particular has been shown to increase predation rates (Vinyard & O'Brien, 1975; Giguere & Northcote, 1987). Second, motion has also been shown to increase

the susceptibility of zooplankton prey to planktivores (Zaret, 1972; Kislalioglu & Gibson, 1976; Eggers, 1977; Zaret, 1980), and thus the level of feeding activity of a zooplankter would be expected to influence predation rates. Reduced feeding would make the zooplankter less conspicuous to visual predators in either case, and would therefore seem to be of adaptive advantage when risk of predation is high. Alternatively, copepods in the presence of fish may experience a reduced level of feeding simply because they spend a larger proportion of their time actively avoiding predators (i.e., swimming) and less time feeding; however, given that prey motion can increase predation rates, a better strategy for the copepods, having sensed the presence of fish initially, may be to remain still and reduce feeding.

Thus, while diel cycles of migration and feeding in zooplankton may have the same ultimate cause or adaptive significance (i.e., predator evasion), the diel feeding cycle is not, as has been previously suggested, simply the result of animals migrating into and out of food rich surface waters. Rather, diel cycles of migration and feeding are separate and independent behaviors (Head et al., 1985; Stearns, 1986), both of which allow animals to reduce their chances of being seen and consumed by visual predators: zooplankton typically reside in darker subsurface waters and feed less actively during the day, which affords the animal reduced threat from visual predators but also has the attendant cost of reduced energy intake; animals then migrate into surface waters to feed more actively only under the cover of darkness, when risk of predation is low. In this way diel cycles of migration and feeding in zooplankton can be viewed as specific examples of the more general phenomenon (Milinski, 1986; Dill, 1987; Sih, 1987) of animals making compromises or trade-offs between time spent foraging and avoiding predators.

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